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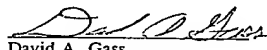
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EXHIBIT

PATENT  
28967/33072

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is being
Alitalo et al.	)	deposited with the United States Postal
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Title: RECEPTOR LIGAND	)	Assistant Commissioner for Patents
Art Unit: 1801	)	Washington, D.C. 20231, on this date:
Examiner: Lathrop, B.	)	Dated: <u>Nov. 26, 1997</u>
	)	
	)	David A. Gass
	)	Registration No. 38,153

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated May 28, 1997, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

Isolation of VEGF-C protein and cDNA

2. The present invention relates generally to a protein ligand for Flt4 receptor tyrosine kinase (VEGFR-3), which our research team has designated "VEGF-C." As taught in Example 14 of the patent application, VEGF-C also stimulates KDR/Flk-1 receptor tyrosine kinase (VEGFR-2). Our research team purified a VEGF-C protein that we discovered in conditioned media from a PC-3 prostatic adenocarcinoma cell line. We demonstrated that this protein bound to the extracellular domain of Flt4 and stimulated Flt4 phosphorylation. (See the patent application at Examples 4-5, for example.) Using SDS polyacrylamide gel electrophoresis, the VEGF-C protein was originally determined to have a molecular weight of about 23 kilodaltons. This measurement is in good

agreement with subsequent measurements of VEGF-C that we have recombinantly expressed in multiple cell lines, where we have determined the molecular weight to be about 21-23 kD.)

3. We sequenced the amino terminus of this purified VEGF-C protein as taught in the patent application in Example 5. (See especially p. 23.) I hereby reaffirm that our sequencing data from this protein is correctly reported in the patent application at p. 23 and in SEQ ID NO: 13.

4. As taught in Examples 6-10 of the patent application, we used the amino terminal amino acid sequence taught in the patent application to obtain a cDNA encoding VEGF-C. A plasmid containing the cDNA that is described in Example 11 of the patent application was deposited with the American Type Culture Collection and accorded ATCC accession number 97231.

5. The patent application describes a partial nucleotide sequence and a 350 amino acid open reading frame of the deposited VEGF-C cDNA. (See SEQ ID NOs: 32 and 33 of the patent application.) In the amendment filed herewith, these sequences have been amended such that the designation of residue "1" therein corresponds with the first residue of VEGF-C purified from PC-3 conditioned medium as described in the patent application. (See also paragraph 3, above.) Amended SEQ ID NOs: 32-33 are attached hereto as Exhibit A. Complete sequencing of the cDNA subsequently demonstrated that the translated open reading frame is actually 419 amino acids: it extends 69 codons upstream of what is reported in SEQ ID NO: 33. Attached hereto as Exhibit B is a 1997 nucleotide sequence of the cDNA that was deposited with the ATCC. Exhibit B also depicts the deduced 419 amino acid open reading frame. These sequences have been added to the patent application as SEQ ID NOs: 44 and 45. I shall use the term "prepro-VEGF-C" herein to refer to a polypeptide consisting of this 419 amino acid sequence.

6. As taught in the patent application (e.g., at p. 11), the carboxyl-terminal amino acid sequences encoded by the VEGF-C cDNA show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring 3 protein (BR3P) sequence that was



known in the art. (See Dignam and Case, *Gene*, 88:133-40 (1990); and Paulsson, *et al.*, *J. Mol. Biol.*, 211:331-49 (1990), both of record and cited in the patent application). The distinctive BR3P cysteine motifs (Cys-Xaa<sub>n</sub>-Cys-Xaa-Cys-Xaa-Cys, wherein Xaa is any residue and n is variable) occur at least four times in the carboxy-terminal portion of VEGF-C (see Cys residues in Exhibit B at positions 280, 291, 293, and 295; positions 304, 315, 317, and 319; positions 328, 339, 341, and 343; and positions 347, 358, 360, and 362).

**VEGF-C processing and determination of  
VEGF-C fragments that bind to Flt4.**

7. The Patent application teaches that the protein encoded by the VEGF-C gene is proteolytically processed, and teaches procedures to characterize this processing, such as analysis using antibodies and pulse-chase experiments. The application further teaches to screen truncated forms of VEGF-C (e.g., deletion fragments) to determine the portions of VEGF-C that are necessary to bind and stimulate Flt4. (See, e.g., pp. 29-30 of the patent application.) Using techniques such as those described at pp. 29-30 of the patent application and mutational analysis, our research team has extensively characterized the processing of human prepro-VEGF-C in mammalian cell lines.

A. Our results from pulse-chase experiments indicate that the apparent first proteolytic processing of human prepro-VEGF-C involves cleavage of a signal peptide of about 31 residues, leaving residues 32-419 (hereinafter "pro-VEGF-C"). Pro-VEGF-C has an apparent molecular weight of about 55-58 kD.

B. We next observed that pro-VEGF-C is cleaved, either intracellularly or at the cell surface, into polypeptides of about 29 kD and about 31-32 kD (when assessed by SDS-PAGE under reducing conditions). The ~32 kD polypeptide binds the extracellular domain of Flt4 receptor tyrosine kinase with high affinity. (See Example 13 of the patent application.) The ~32 kD polypeptide was purified with immunoaffinity chromatography using an anti-VEGF-C antibody. The amino-terminus of

this purified polypeptide was determined to correspond to position 32 of the sequence shown in Exhibit B. Thus, the ~32 kD polypeptide represents the amino-terminal product of this proteolytic cleavage. Sequencing of the ~29 kD polypeptide indicated that cleavage occurred after amino acid 227 of the 419 amino acid sequence depicted in Exhibit B. (Amino acid 227 corresponds to residue 125 of SEQ ID NO: 33 in the patent application (Exhibit A).) This carboxy-terminal fragment of about 29 kD presumably includes residues 228-419 of the sequence depicted in Exhibit B (residues 126-317 of SEQ ID NO: 33). Thus, the ~29 kD polypeptide includes all of the Balbiani ring 3 protein cysteine motifs of VEGF-C (see paragraph 6 above). These results indicate that polypeptide fragments of the sequences depicted in Exhibits A or B that lack any domain having cysteine motifs of a Balbiani ring 3 protein (e.g., that lack the ~29 kD carboxy-terminal fragment) remain capable of binding with the extracellular domain of Flt4.

C. We also have observed forms of VEGF-C that reflect further proteolytic processing at the amino terminus. For the purpose of this declaration, I shall collectively refer to forms of VEGF described below as "mature VEGF-C."

- i. As indicated in paragraph 3, above, VEGF-C isolated from conditioned medium of PC-3 cells has an amino terminus corresponding to amino acid 103 in Exhibit B (i.e., amino acid 1 of SEQ ID NO: 33 (Exhibit A)).
- ii. We have sequenced VEGF-C that was recombinantly expressed in 293-EBNA cells (as described in Example 11 of the patent application) and determined that the amino terminus of this form corresponds with position 112 of the sequence shown in Exhibit B (i.e., position 10 of SEQ ID NO: 33 (Exhibit A)).

8. Our research team modified the human VEGF-C cDNA to recombinantly produce a fragment consisting of amino acids 104-213 of the 419 amino acid polypeptide in yeast (i.e., residues 2-111 of SEQ ID NO: 33). This fragment was shown to bind Flt4 and stimulate phosphorylation of both Flt4 (VEGFR-3) and KDR (VEGFR-2). In another experiment, a fragment lacking residues 1-112 of the 419 amino acid polypeptide retained receptor binding activity.

9. Collectively, the experimental results described in the preceding paragraphs indicate that polypeptides lacking amino acids 1-112 and 214-419 of the 419 residue amino acid sequence shown in Exhibit B retain Flt4 binding and stimulating activities. Stated differently, we have experimental evidence to indicate that a polypeptide corresponding to positions 11-112 of SEQ ID NO: 33 will retain Flt4 binding and stimulating activities. Moreover, one skilled in the art understands from the patent application how to perform receptor binding and phosphorylation assays, to localize further the portion of SEQ ID NO: 33 that is required for activity.

The application enables one to obtain  
VEGF-C-encoding cDNAs from non-human sources

10. I infer from page 5 of the Office action that the Patent Office has rejected a claim of the application in part because of the lack of a claim limitation with respect to the source animal for VEGF-C. This section of the declaration provides evidence that the teachings in the patent application of a human VEGF-C cDNA, combined with the teachings that VEGF-C protein binds Flt4 (VEGFR-3) and VEGFR-2, enable one to obtain VEGF-C-encoding cDNAs from non-human sources.

11. To clone a murine VEGF-C cDNA, approximately  $1 \times 10^6$  bacteriophage lambda clones of a commercially-available 12 day mouse embryonal cDNA library (lambda EXlox library, Novagen, catalog number 69632-1), were screened with a radiolabeled fragment of human VEGF-C cDNA containing nucleotides 495 to 1661 of the nucleotide sequence shown in Exhibit B. One positive clone was isolated.

12. A 1323 bp *EcoRI/HindIII* fragment of the insert of the isolated mouse cDNA clone was subcloned into the corresponding sites of the pBluescript SK+ vector (Stratagene) and sequenced. The cDNA sequence of this clone was homologous to the human VEGF-C sequence reported herein, except that about 710 bp of 5'-end sequence present in the human clone was not present in the mouse clone.

13. For further screening of mouse cDNA libraries, a *HindIII-BstXI* (*HindIII* site is from the pBluescript SK+ polylinker) fragment of 881 bp from the coding region of the mouse cDNA clone was radiolabeled and used as a probe to screen two additional mouse cDNA libraries. Two additional cDNA clones from an adult mouse heart ZAP II cDNA library (Stratagene, catalog number 936306) were identified. Three additional clones also were isolated from a mouse heart 5'-stretch-plus cDNA library in  $\lambda$ gt11 (Clontech Laboratories, Inc., catalog number ML5002b). Of the latter three clones, one was found to contain an insert of about 1.9 kb. The insert of this cDNA clone was subcloned into *EcoRI* sites of pBluescript SK+ vector and both strands of this clone were completely sequenced, resulting in the nucleotide and deduced amino acid sequences shown in Exhibit C. It is expected that the mouse VEGF-C polypeptide depicted in Exhibit C is processed into a mature mouse VEGF-C protein, in a manner analogous to the processing of the human prepro-VEGF-C.

14. The foregoing results demonstrate the utility of human VEGF-C-encoding polynucleotides of the invention for identifying and isolating polynucleotides encoding other non-human mammalian VEGF-C proteins. Such identified and isolated polynucleotides, in turn, can be expressed (using procedures similar to those described in the patent application for human VEGF-C) to produce recombinant polypeptides corresponding to non-human mammalian forms of VEGF-C.

15. The identity of the mouse protein as VEGF-C was confirmed by recombinantly expressing the above-described mouse cDNA, and analyzing the expressed proteins.

A. The 1.8 kb mouse VEGF-C cDNA was cloned as an *EcoRI* fragment into the retroviral expression vector pBabe-puro containing the SV40 early promoter region [Morgenstern *et al.*, *Nucl. Acids Res.*, 18:3587-3595 (1990)], and transfected into the Bosc23 packaging cell line [Pearret *et al.*, *Proc. Natl. Acad. Sci. (USA)*, 90:8392-8396 (1994)] by the calcium-phosphate precipitation method. For comparison, Bosc23 cells also were transfected with the previously-described human VEGF-C construct in the pREP7 expression vector. The expressed proteins were immunoprecipitated with polyclonal antibodies raised against mature human VEGF-C.

B. Immunoprecipitation of VEGF-C from media of transfected and metabolically-labelled cells revealed bands of approximately  $30\text{-}32 \times 10^3$  M<sub>r</sub> (a doublet) and  $22\text{-}23 \times 10^3$  M<sub>r</sub> in 12.5% SDS-PAGE. These bands were not detected in samples from nontransfected or mock-transfected cells. These results demonstrate that antibodies raised against human VEGF-C recognize the corresponding mouse protein.

C. For receptor binding experiments, 1 ml aliquots of media from metabolically-labelled Bosc23 cells were incubated with VEGFR-3 extracellular domain, covalently coupled to sepharose, for 4 hours at 4°C with gentle mixing. (See Examples 4 and 5 in the patent application.) The sepharose beads were washed four times with ice-cold phosphate buffered saline (PBS), and the samples were analyzed by gel electrophoresis as described in Joukov *et al.*, *EMBO J.*, 15:290-298 (1996).

D. Similar  $30\text{-}32 \times 10^3$  M<sub>r</sub> doublet and  $22\text{-}23 \times 10^3$  M<sub>r</sub> polypeptide bands were obtained in the receptor binding assay as compared to the immunoprecipitation assay. In additional experiments, mouse VEGF-C appeared to be a potent inducer of VEGFR-3 autophosphorylation, too. Thus, the putative mouse VEGF-C binds and stimulates human VEGFR-3, confirming its identity. The slightly faster mobility of the mouse VEGF-C polypeptides that was observed may be caused by the four amino acid

residue difference observed in sequence analysis (residues H88-E91).  
Murine VEGF-C appeared to bind VEGFR-2 with lower affinity.

16. The human VEGF-C cDNA also was used to design probes for successfully isolating a quail VEGF-C cDNA from a quail cDNA library. A fragment of the human VEGF-C cDNA comprising nucleotides 495-1661 of Exhibit B was obtained by PCR amplification, cloned into the pCRII vector (Invitrogen) according to the manufacturer's instructions, and amplified. The insert was isolated by *Eco* RI digestion and preparative gel electrophoresis and then labelled using radioactive dCTP and random priming. A cDNA library made from quail embryos of stage E-4 in pcDNA-1 vector (Invitrogen) was then screened using this probe. About 200,000 colonies were plated and filter replicas were hybridized with the radioactive probe under reduced stringency conditions (washes at 42°C with a wash solution comprising 2x SSC/0.1% SDS). Nine positive clones were identified and secondarily plated. Two of the nine clones hybridized in secondary screening. The purified clones (clones 1 and 14) had approximately 2.7 kb *Eco* RI inserts. Both clones were amplified and then sequenced using the T7 and SP6 primers (annealing to the vector). In addition, an internal *Sph* I restriction endonuclease cleavage site was identified about 1.9 kb from the T7 primer side of the vector and used for subcloning 5'- and 3'- *Sph* I fragments, followed by sequencing from the *Sph* I end of the subclones. The sequences obtained were identical from both clones and showed a high degree of similarity to the human VEGF-C coding region. Subsequently, walking primers were made in both directions and double-stranded sequencing was completed for 1743 base pairs, including the full-length open reading frame.

17. The cDNA sequence obtained includes a long open reading frame and 5' untranslated region. The DNA and deduced amino acid sequences for the quail cDNA are set forth in Exhibit D. Studies performed with the putative quail VEGF-C cDNA have shown that its protein product is secreted from transfected cells and interacts with avian VEGFR-3 and VEGFR-2, further confirming the conclusion that the cDNA encodes a quail VEGF-C protein.

18. As shown in Exhibit E, the human, murine, and avian (quail) VEGF-C precursor amino acid sequences share a significant degree of conservation. This high degree of homology confirms the likelihood of success of attempts to isolate VEGF-C encoding sequences from other species, especially vertebrate species, and more particularly mammalian and avian species, using human VEGF-C-encoding polynucleotides taught in the patent application as probes and using standard molecular biological techniques. The identity of putative VEGF-C-encoding cDNAs is confirmed using receptor binding studies such as the studies described in the patent application.

Certification

19. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

December 20, 1997

Date

Jan Alitalo

Kari Alitalo

EXHIBIT A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAAC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	-33 -30	
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA		102
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln		
	-25 -20 -15	
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA		150
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile		
	-10 -5 1 5	
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT		198
Lys Phe Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
	10 15 20	
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT		246
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
	25 30 35	
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA		294
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Lys Pro Pro		
	40 45 50	
TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAT AGT GAG GGG CTG		342
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
	55 60 65	
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA		390
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
	70 75 80 85	
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT		438
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
	90 95 100	
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA		486
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
	105 110 115	
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG		534
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
	120 125 130	
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT		582
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
	135 140 145	
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT		630
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
	150 155 160 165	
GCT GGA GAT GAC TCA ACA GAT GGA TTC CAT GAC ATC TGT GGA CCA AAC		678
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
	170 175	
AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT		726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu		
	185 190 195	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC		774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys		
	200 205 210	





Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser  
 100 105 110  
 Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu  
 115 120 125  
 Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr  
 130 135 140  
 Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp  
 145 150 155  
 Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His  
 160 165 170 175  
 Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys  
 180 185 190  
 Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu  
 195 200 205  
 Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro  
 210 215 220  
 Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys  
 225 230 235  
 Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys  
 240 245 250 255  
 Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly  
 260 265 270  
 Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr  
 275 280 285  
 Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val  
 290 295 300  
 Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser  
 305 310 315

EXHIBIT B

CCCCCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGGCC	60
CTCGCTTCAC CTGCGGGCT CGGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC	120
TTTACCTGA CACCCGCGC CTTCCTCCGG CACTGGCTGG GAGGCGGCC TGCAAAAGTTG	180
GGAAACGGGA GCGCCGAGCC CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCGCG	240
GAGGAGCCCG GGGGAGAGGG ACCAGAGGG GCGCGCGGCC TCGCAGGGGC CCGCGCGCCC	300
CCACCCCTGC CCGCGCCAGC GGACCGGTCC CCCACCCCG GTCTTCCAC C ATG CAC Met His 1	357
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu 5 10 15	405
CTC CGG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC TTC GAG TCC Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser 20 25 30	453
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala 35 40 45 50	501
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Val 55 60 65	549
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys 70 75 80	597
TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn 85 90 95	645
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr 100 105 110	693
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln 115 120 125 130	741
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val 135 140 145	789
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT Ala Thr Asn Thr Phe Phe Lys Pro Cys Val Ser Val Tyr Arg Cys 150 155 160	837
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981

TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA	1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg	
215 220 225	
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC	1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr	
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT	1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT	1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC	1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC	1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA	1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	
310 315 320	
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA	1365
Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr	
325 330 335	
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT	1413
Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn	
340 345 350	
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG	1461
Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu	
355 360 365 370	
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG	1509
Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg	
375 380 385	
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT	1557
Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser	
390 395 400	
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGG AAA AGA CCA CAA ATG	1605
Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met	
405 410 415	
AGC TAAGATTGTA CTGTTTCCCA GTTCATCGAT TTTCTATTAT GGAAAACTGT	1658
Ser	
GTGGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCTGGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAAAACAGCC AAGATTTCCT	1838
TCTGTGTAAT TCTTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTTCT	1898
GCATTCAITT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCAARAATA TGTTTAAAT AAAATGAAAA TTGTATTAT	1997

# EXHIBIT C

## Mouse VEGF-C cDNA and deduced amino acid sequence

CGGGCCCGCT	CGACGCARAA	GTTCGAGGCC	GCCGAGTCCC	GGGAGACGCT	CGCCCAGGGG	60
GGTCCCCGGG	AGGAAACCAC	GGGACAGGGA	CCAGGAGAGG	ACCTCAGCCT	CACGCCCCAG	120
CCTGCGCCAG	CCAACGGACC	GGCCTCCCTG	CTCCCGSTCC	ATCCACC	ATG CAC TTG Met His Leu 1	176
CTG TGC TFC TTG TCT CTG GCG TGT TCC CTG CTC GCC GCT GCG CTG ATC	Leu Cys Phe Leu Ser Leu Ala Cys Ser Leu Leu Ala Ala Ala Leu Ile	5 10 15	224			
CCC AGT CCG CGC GAG GCG CCC GCC ACC GTC GCC GCC TTC GAG TCG GGA	Pro Ser Pro Arg Glu Ala Glu Pro Ala Thr Val Ala Ala Phe Glu Ser Gly	20 25 30 35	272			
CTG GGC TTC TCG GAA GCG GAG CCC GAC GGG GGC GAG GTC AAG GCT TTT	Leu Gly Phe Ser Glu Ala Glu Pro Asp Gly Gly Glu Val Lys Ala Phe	40 45 50	320			
GAA GGC AAA GAC CTG GAG GAG CAG TTG CCG TCT GTG TCC AGC GTA GAT	Glu Cys Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Val Asp	55 60 65	368			
GAG CTG ATG TCT GTC CTG TAC CCA GAC TAC TGG AAA ATG TAC AAG TGC	Glu Leu Met Ser Val Leu Tyr Pro Asp Tyr Trp Lys Met Tyr Lys Cys	70 75 80	416			
CAG CTG CGG AAA GGC GGC TGG CAG CAG CCC ACC CTC AAT ACC AGG ACA	Gln Leu Arg Lys Gly Gly Trp Gln Gln Pro Thr Leu Asn Thr Arg Thr	85 90 95	464			
GGG GAC AGT GTA AAA TTT GCT GCT GCA CAT TAT AAC ACA GAG ATC CTG	Gly Asp Ser Val Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu	100 105 110 115	512			
AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGT GAG	Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu	120 125 130	560			
GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GCA GCC ACA AAC ACC TTC	Val Cys Ile Asp Val Gly Lys Glu Phe Gly Ala Ala Thr Asn Thr Phe	135 140 145	608			
TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT GGG GGT TGC TGC AAC	Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn	150 155 160	656			
AGC GAG GGG CTG CAG TGC ATG AAC ACC AGC ACA GGT TAC CTC AGC AAG	Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Gly Tyr Leu Ser Lys	165 170 175	704			
ACG TTG TTT GAA ATT ACA GTG CCT CTC TCA CAA GGC CCC AAA CCA GTC	Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val	180 185 190 195	752			
ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGG TGC ATG TCT AAA CTG	Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu	200 205 210	800			
GAT GTT TAC AGA CAA GTT CAT TCA ATT ATT AGA CGT TCT CTG CCA GCA	Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala	215 220 225	848			

ACA TTA CCA CAG TGT CAG GCA GCT AAC AAG ACA TGT CCA ACA AAC TAT Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr 230 235 240	896
GTG TGG AAT AAC TAC ATG TGC CGA TGC CTG GCT CAG GAT TTT ATC Val Trp Asn Asn Tyr Met Cys Arg Cys Leu Ala Gln Asp Phe Ile 245 250 255	944
TTT TAT TCA AAT GTT GAA GAT GAC TCA ACC AAT GGA TTC CAT GAT GTC Phe Tyr Ser Asn Val Glu Asp Asp Ser Thr Asn Gly Phe His Asp Val 260 265 270 275	992
TGT GGA CCC AAC AAG GAG CTG GAT GAA GAC ACC TGT CAG TGT GTC TGC Cys Gly Pro Asn Lys Glu Leu Asp Glu Asp Thr Cys Gln Cys Val Cys 280 285 290	1040
AAG GGG GGG CTT CGG CCA TCT AGT TGT GGA CCC CAC AAA GAA CTA GAT Lys Gly Gly Leu Arg Pro Ser Ser Cys Gly Pro His Lys Leu Asp 295 300 305	1088
AGA GAC TCA TGT CAG TGT GTC TGT AAA AAC AAA CTT TTC CCT AAT TCA Arg Asp Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Asn Ser 310 315 320	1136
TGT GGA GCC AAC AGG GAA TTT GAT GAG AAT ACA TGT CAG TGT GTA TGT Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys 325 330 335	1184
AAA AGA ACG TGT CCA AGA AAT CAG CCC CTG AAT CCT GGG AAA TGT GCC Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala 340 345 350 355	1232
TGT GAA TGT ACA GAA AAC ACA CAG AAG TGC TTC CTT AAA GGG AAG AAG Cys Glu Cys Thr Glu Asn Thr Gln Lys Cys Phe Leu Lys Gly Lys Lys 360 365 370	1280
TTC CAC CAT CAA ACA TGC AGT TGT TAC AGA AGA CCG TGT GCG AAT CGA Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Ala Asn Arg 375 380 385	1328
CTG AAG CAT TGT GAT CCA GGA CTG TCC TTT AGT GAA GAA GTA TGC CGC Leu Lys His Cys Asp Pro Gly Leu Ser Phe Ser Glu Glu Val Cys Arg 390 395 400	1376
TGT GTC CCA TCG TAT TGG AAA AGG CCA CAT CTG AAC TAAGATCAT Cys Val Pro Ser Tyr Trp Lys Arg Pro His Leu Asn 405 410 415	1422
CCAGTTTTC A GTCAGTCACA GTCATTACT CTCITGAAGA CTGTTGGAAC AGCACTTAGC	1482
ACTGTCTATG CACAGAAAGA CTCGTGIGGA CCACATGGTA ACAGAGGCCC AAGTCGTGT	1542
TTATTGAACC ATGTGGATTA CTGCGGGAGA GGACTGGCAC TCATGTGCA AAAAAACCTC	1602
TTCAAGACT GGTTTTCTGC CAGGGACCAG ACAGCTGAGG TTTTCTCTT GTGATTAA	1662
AAAAGAATGA CTATATAATT TATTTCCACT AAAAATATTG TTCCTGCATT CATTTTATA	1722
GCAATAACAA TTGTAAGC TCAGTGTGAT CAGTATTTTT ATAACATGCA AACTATGTT	1782
TAAAAA TGA AAT TGT ATTATAAAAA AAAAAAAAAA AAAAAAAAAA GCTT	1836

EXHIBIT D

Quail VEGF-C

GCCCCCGCCG AGCGCTCCGC GCGCAGCCGC CGGGCCGGGC CGGCCCGCGS AGGGCGCGCT	60
GCGAGCGGCC ACTGGGTCTT GCTTCCCTCC TTCTCTTCCC TCTCTCTCTT CCTCTCTCTC	120
TCTGCGCTTT CCACCGCTCC CGAGCGAGCG CACGCTCGGA TGTCGGTTT CCTGGTGGGT	180
TTTTTACCTG GCAAAGTCCG GATRACTTCG GTGAGAATTT GCAAAGAGGC TGGGAGCTCC	240
CCTGCAGCGS TCTGGGAGCT GCTGCCGCGG TCGCATCTTC TCCATCCCGC GGATTTTACT	300
GCCTTGGATA TTGCGAGGGG AGGGAGGGGG GTGAGGACAG CAAAAGAAA GGGGTGGGGG	360
GGGGGAGAGA AAAGGAAAAG AAGGAGCCTC GGAATTGTGC CCGCATTCCT CGCCTGCCCC	420
GCGGCCCCCC TCCGCTCTGC CATCTCCGCA CA ATG CAC TTG CTG GAG ATG CTC	473
Met His Leu Leu Glu Met Leu	
1 5	
TCC CTG GGC TGC TGC CTC GCT GCT GGC GCC GTG CTC CTG GGA CCC CGG	521
Ser Leu Gly Cys Cys Leu Ala Ala Gly Ala Val Leu Gly Pro Arg	
10 15 20	
CAG CCG CCC GTC GCC GCC GCC TAC GAG TCC GGG CAC GGC TAC TAC GAG	569
Gln Pro Pro Val Ala Ala Ala Tyr Glu Ser Gly His Gly Tyr Glu	
25 30 35	
GAG GAG CCC GGT GCC GGG GAA CCC AAG GCT CAT GCA AGC AAA GAC CTG	617
Glu Glu Pro Gly Ala Gly Glu Pro Lys Ala His Ala Ser Lys Asp Leu	
40 45 50 55	
GAA GAG CAG TTG CGA TCT CTG TCC AGT GTG GAT GAA CTC ATG ACA GTA	665
Glu Glu Gln Leu Arg Ser Val Ser Ser Val Asp Glu Leu Met Thr Val	
60 65 70	
CTT TAC CCA GAA TAC TGG AAA ATG TTC AAA TGT CAG TTG AGG AAA GGA	713
Leu Tyr Pro Glu Tyr Trp Lys Met Phe Lys Cys Gln Leu Arg Lys Gly	
75 80 85	
GGT TGG CAA CAC AAC AGG GAA CAC TCC AGC TCT GAT ACA AGA TCA GAT	761
Gly Trp Gln His Asn Arg Glu His Ser Ser Ser Asp Thr Arg Ser Asp	
90 95 100	
GAT TCA TTG AAA TTT GCC GCA GCA CAT TAT AAT GCA GAG ATC CTG AAA	809
Asp Ser Leu Lys Phe Ala Ala Ala His Tyr Asn Ala Glu Ile Leu Lys	
105 110 115	
AGT ATT GAT ACT GAA TGG AGA AAA ACC CAG GGC ATG CCA CGT GAA GTG	857
Ser Ile Asp Thr Glu Trp Arg Lys Thr Gln Gly Met Pro Arg Glu Val	
120 125 130 135	
TGT GTG GAT TTG GGG AAA GAG TTT GGA GCA ACT ACA AAC ACC TTC TTT	905
Cys Val Asp Leu Gly Lys Phe Gly Ala Thr Thr Asn Thr Phe Phe	
140 145 150	
AAA CCC CCG TGT GTA TCC ATC TAC AGA TGT GGA GGT TGC TGC AAT AGT	953
Lys Pro Pro Cys Val Ser Ile Tyr Arg Cys Gly Cys Cys Asn Ser	
155 160 165	
GAA GGA CTC CAG TGT ATG AAT ATC AGC ACA AAT TAC ATC AGC AAG ACA	1001
Glu Gly Leu Gln Cys Met Asn Ile Ser Thr Asn Tyr Ile Ser Lys Thr	
170 175 180	

TTG TTT GAG ATT ACA GTG CCT CTC TCT CAT GGC CCC AAA CCT GTA ACA Leu Phe Glu Ile Thr Val Pro Leu Ser His Gly Pro Lys Pro Val Thr 185 190 195	1049
GTC AGT TTT GCC AAT CAC ACG TCC TGC CGA TGC ATG TCT AAG TTG GAT Val Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp 200 205 210 215	1097
GTT TAC AGA CAA GTT CAT TCT ATC ATA AGA CGT TCC TTG CCA GCA ACA Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr 220 225 230	1145
CAA ACT CAG TGT CAT GTG GCA AAC AAG ACC TGT CCA AAA AAT CAT GTC Gln Thr Gln His Val Ala Asn Lys Thr Cys Pro Lys Asn His Val 235 240 245	1193
TGG AAT AAT CAG ATT TGC AGA TGC TTA GCA CAG CAC GAT TTT GGT TTC Trp Asn Asn Gln Ile Cys Arg Cys Leu Ala Gln His Asp Phe Gly Phe 250 255 260	1241
TCT TCT CAC CTT GGA GAT TCT GAC ACA TCT GAA GGA TTC CAT ATT TGT Ser Ser His Leu Gly Asp Ser Asp Thr Ser Gly Phe His Ile Cys 265 270 275	1289
GGG CCC AAC AAA GAG CTG GAT GAA GAA ACC TGT CAA TGC GTC TGC AAA Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Lys 280 285 290 295	1337
GGA GGT GTG CGG CCC ATA AGC TGT GGC CCT CAC AAA GAA CTA GAC AGG Gly Gly Val Arg Gln Pro Ile Ser Cys Gly Pro His Lys Glu Leu Asp Arg 300 305 310	1385
GCA TCA TGT CAG TGC ATG TGC AAA AAC AAA CTG CTC CCC AGT TCC TGT Ala Ser Cys Gln Cys Met Cys Lys Asn Lys Leu Leu Pro Ser Ser Cys 315 320 325	1433
GGG CCT AAC AAA GAA TTT GAT GAA GAA AAG TGC CAG TGT GTA TGT AAA Gly Pro Asn Lys Glu Phe Asp Glu Glu Lys Cys Gln Cys Val Cys Lys 330 335 340	1481
AAG ACC TGT CCC AAA CAT CAT CCA CTA AAT CCT GCA AAA TGC ATC TGC Lys Thr Cys Pro Lys His His Pro Leu Asn Pro Ala Lys Cys Ile Cys 345 350 355	1529
GAA TGT ACA GAA TCT CCC AAT AAA TGT TTC TTA AAA GGA AAA AGA TTT Glu Cys Thr Glu Ser Pro Asn Lys Cys Phe Leu Lys Gly Lys Arg Phe 360 365 370 375	1577
CAT CAC CAG ACA TGC AGT TGT TAC AGA CCA CCA TGT ACA GTC CGA ACG His His Gln Thr Cys Ser Cys Tyr Arg Pro Pro Cys Thr Val Arg Thr 380 385 390	1625
AAA CGC TGT GAT GCT GGA TTT CTG TTA GCT GAA GAA GTG TGC CGC TGT Lys Arg Cys Asp Ala Gly Phe Leu Leu Ala Glu Glu Val Cys Arg Cys 395 400 405	1673
GTA CGC ACA TCT TGG AAA AGA CCA CTT ATG AAT TAAGCGAAGA AAGCACTACT Val Arg Thr Ser Trp Lys Arg Pro Leu Met Asn 410 415	1726
CGCTATATAG TGTCG	1741



# Notice of Allowability

Application No.  
08/510,133

Av: int(s)

ALITALO et al.

Examiner

Christine Saoud

Group Art Unit

1647

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance and Issue Fee Due or other appropriate communication will be mailed in due course.

☒ This communication is responsive to amendment of 26 July 2000

☒ The allowed claim(s) is/are 29-57, renumbered as 1-29

☐ The drawings filed on \_\_\_\_\_ are acceptable.

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\* Certified copies not received: \_\_\_\_\_

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" of this Office action. Failure to timely comply will result in ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.

☒ Applicant MUST submit NEW FORMAL DRAWINGS

☐ because the originally filed drawings were declared by applicant to be informal.

☒ including changes required by the Notice of Draftsperson's Patent Drawing Review, PTO-948, attached hereto or to Paper No. 7.

☐ including changes required by the proposed drawing correction filed on \_\_\_\_\_, which has been approved by the examiner.

☐ including changes required by the attached Examiner's Amendment/Comment.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the reverse side of the drawings. The drawings should be filed as a separate paper with a transmittal letter addressed to the Official Draftsperson.

☐ Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Any response to this letter should include, in the upper right hand corner, the APPLICATION NUMBER (SERIES CODE/SERIAL NUMBER). If applicant has received a Notice of Allowance and Issue Fee Due, the ISSUE BATCH NUMBER and DATE of the NOTICE OF ALLOWANCE should also be included.

## Attachment(s)

☐ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 18

☒ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

☐ Interview Summary, PTO-413

☐ Examiner's Amendment/Comment

☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material

☐ Examiner's Statement of Reasons for Allowance

CHRISTINE SAOUD  
PATENT EXAMINER

*Christine Saoud*

## EXHIBIT E

## VEGF-C alignment

	1				50
Hum	MHLGFFSVA	CSLLAAALP	GPREAPAAA	AFESGLDLS	AEPDAGEATA
Mou	MHLGFFSLA	CSLLAAALP	SPREAPATVA	AFESGLGFSE	AEPDGGEVKA
Qua	MHLLEMLSLG	CCLAAGAVLL	GPRQPPVA.A	AYESGHGYE	EEPGAGEPKA
	51				100
Hum	YASKDLEEQL	RSVSSVDELM	TVLYPEYWK	YKQLRKGGW	QHNREQANLN
Mou	FEGKDLEEQL	RSVSSVDELM	SVLYPDYWK	YKQLRKGGW	Q....QPTLN
Qua	HASKDLEEQL	RSVSSVDELM	TVLYPEYWK	FKQLRKGGW	QHNREHSSD
	101				150
Hum	SRTEETIKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVCI	DV GKEFGVATNT
Mou	TRTGDSVKFA	AAHYNTEILK	SIDNEWRTQ	CMPEVCI	DV GKEFGAATNT
Qua	TRSDDSLKFA	AAHYNAEILK	SIDTEWRTQ	GMPEVCDL	GKEFGATTNT
	151				200
Hum	FFKPPCVSVY	RCGGCCNSEG	LQCHNTSTY	LSKTLFEITV	PLSQGPKPVT
Mou	FFKPPCVSVY	RCGGCCNSEG	LQCHNTSTY	LSKTLFEITV	PLSQGPKPVT
Qua	FFKPPCVSIY	RCGGCCNSEG	LQCHNISTNY	LSKTLFEITV	PLSHGPKPVT
	201				250
Hum	ISFANETSCR	CMKLDVYRQ	VHSIIRSLP	ATLPQCOAAN	KTCPTNYMWN
Mou	ISFANETSCR	CMKLDVYRQ	VHSIIRSLP	ATLPQCOAAN	KTCPTNYMWN
Qua	VSFANETSCR	CMKLDVYRQ	VHSIIRSLP	ATQTQCHVAN	KTCPKNHVMN
	251				300
Hum	NHICRCLAQE	DFMFSSDAGD	DSTDGFHDIC	GNPKELDEET	CQCVCRAGLR
Mou	NYMCRCLAAQ	DFIFYGNVED	DSTNGFHDVC	GNPKELDEET	CQCVCCKGGLR
Qua	NQICRCLAQH	DFGFSHGLGD	SDTSEGFHIC	GNPKELDEET	CQCVCCKGVR
	301				350
Hum	PASCGPHKEL	DRNSCQCVC	NKLFPSCGA	NREFDENTCQ	CVCKRTCPRN
Mou	PASCGPHKEL	DRNSCQCVC	NKLFPSCGA	NREFDENTCQ	CVCKRTCPRN
Qua	PISCGPHKEL	DRASCQCMCK	NKLLPSSCGP	NKEFDEEKQ	CVCKKTCPKH
	351				400
Hum	QPLNPGKAC	ECTESPQKCL	LKGKKFHHQT	CSCYRRPCTN	RQKACEPGFS
Mou	QPLNPGKAC	ECTENTQKCF	LKGKKFHHQT	CSCYRRPCAN	RLXHCDPGLS
Qua	HPLNPAKAC	ECTESPQKCF	LKGKRFHHQT	CSCYRRPCTV	RTKRCADAGL
	401		420		
Hum	YSEEVCRCPV	SYWKRPM*			
Mou	FSEEVCRCPV	SYWKRPHLN*			
Qua	LAEEVCRCPV	TSWKRPLMN*			



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

NOTICE OF ALLOWANCE AND ISSUE FEE DUE

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
First Named Applicant				
TITLE OF INVENTION				

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT.  
PROSECUTION ON THE MERITS IS CLOSED.**

**THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS  
APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.**

**HOW TO RESPOND TO THIS NOTICE:**

I. Review the SMALL ENTITY status shown above.  
If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

- A. If the status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or
- B. If the status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:

- A. Pay FEE DUE shown above, or
- B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

- II. Part B-Issue Fee Transmittal should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by charge to deposit account, Part B Issue Fee Transmittal should be completed and returned. If you are charging the ISSUE FEE to your deposit account, section "4b" of Part B-Issue Fee Transmittal should be completed and an extra copy of the form should be submitted.
- III. All communications regarding this application must give application number and batch number.  
Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.**

## NOTICE OF DRAFTSPERSON'S PATENT DRAWING REVIEW

PTO Draftpersons review all originally filed drawings regardless of whether they are designated as formal or informal. Additionally, patent Examiners will review the drawings for compliance with the regulations. Direct telephone inquiries concerning this review to the Drawing Review Branch, 703-305-8404.

The drawings filed (insert date) 02/01/95 are  
 A. ☒ not objected to by the Draftperson under 37 CFR 1.84 or 1.152.  
 B. ☒ objected to by the Draftperson under 37 CFR 1.84 or 1.152 as indicated below. The Examiner will require submission of new, corrected drawings when necessary. Corrected drawings must be submitted according to the instructions on the back of this Notice.

1. DRAWINGS. 37 CFR 1.84(a): Acceptable categories of drawings:  
 Black ink. Color.

- ☐ Not black solid lines. Fig(s) \_\_\_\_\_  
☐ Color drawings are not acceptable until petition is granted.  
 Fig(s) \_\_\_\_\_

2. PHOTOGRAPHS. 37 CFR 1.84(b) (3 SETS REQUIRED)  
☒ Photographs are not acceptable until petition is granted.

- Fig(s) 4-8, 11-12  
☒ Photographs not properly mounted (must use bristol board or photographic double-weight paper). Fig(s) 4-8, 11-12  
☒ Poor quality (half-tone). Fig(s) 4-8, 11-12

## 3. GRAPHIC FORMS. 37 CFR 1.84(d)

- ☐ Chemical or mathematical formula not labeled as separate figure.  
 Fig(s) \_\_\_\_\_  
☐ Group of waveforms not presented as a single figure, using common vertical axis with time extending along horizontal axis.  
 Fig(s) \_\_\_\_\_  
☐ Individuals waveform not identified with a separate letter designation adjacent to the vertical axis. Fig(s) \_\_\_\_\_

## 4. TYPE OF PAPER. 37 CFR 1.84(e)

- ☐ Paper not flexible, strong, white, smooth, nonshiny, and durable.  
 Sheet(s) \_\_\_\_\_  
☐ Erasures, alterations, overwritings, interlineations, cracks, creases, and folds copy machine marks not accepted. Fig(s) \_\_\_\_\_  
☐ Mylar, velum paper is not acceptable (too thin). Fig(s) \_\_\_\_\_

## 5. SIZE OF PAPER. 37 CFR 1.84(f): Acceptable sizes:

- 21.6 cm. by 35.6 cm. (8 1/2 by 14 inches)  
 21.6 cm. by 33.1 cm. (8 1/2 by 13 inches)  
 21.6 cm. by 27.9 cm. (8 1/2 by 11 inches)  
 21.0 cm. by 29.7 cm. (DIN size A4)  
☐ All drawing sheets not the same size. Sheet(s) \_\_\_\_\_  
☐ Drawing sheet not an acceptable size. Sheet(s) \_\_\_\_\_

## 6. MARGINS. 37 CFR 1.84(g): Acceptable margins:

## Paper size

21.6 cm. X 35.6 cm. (8 1/2 X 14 inches)	21.6 cm. X 33.1 cm. (8 1/2 X 13 inches)	21.6 cm. X 29.7 cm. (8 1/2 X 11 inches)	(DIN Size A4)
T 3.1 cm. (1 1/8")	2.5 cm. (1")	2.5 cm. (1")	2.5 cm.
L .64 cm. (1/16")	.64 cm. (1/16")	.64 cm. (1/16")	2.5 cm.
B .64 cm. (1/16")	.64 cm. (1/16")	.64 cm. (1/16")	1.5 cm.
R .64 cm. (1/16")	.64 cm. (1/16")	.64 cm. (1/16")	1.0 cm.

Margins do not conform to chart above.

Sheet(s) FIG. 2, 5, 10  
☒ Top (T) ☒ Left (L) ☐ Right (R) ☐ Bottom (B)

## 7. VIEWS. 37 CFR 1.84(h)

REMINDER: Specification may require revision to correspond to drawing changes.

- ☐ All views not grouped together. Fig(s) \_\_\_\_\_  
☐ Views connected by projection lines or lead lines.  
 Fig(s) \_\_\_\_\_  
☐ Partial views. 37 CFR 1.84(h) 2

☐ View and enlarged view not labeled separately or properly.

Fig(s) \_\_\_\_\_  
☐ Sectional views. 37 CFR 1.84 (h) 3  
☐ Hatching not indicated for sectional portions of an object.

Fig(s) \_\_\_\_\_  
☐ Cross section not drawn same as view with parts in cross section with regularly spaced parallel oblique strokes. Fig(s) \_\_\_\_\_

## 8. ARRANGEMENT OF VIEWS. 37 CFR 1.84(i)

☐ Words do not appear on a horizontal, left-to-right fashion when page is either upright or turned so that the top becomes the right side, except for graphs. Fig(s) \_\_\_\_\_

## 9. SCALE. 37 CFR 1.84(k)

☐ Scale not large enough to show mechanism with crowding when drawing is reduced in size to two-thirds in reproduction.

Fig(s) \_\_\_\_\_  
☐ Indication such as "actual size" or scale 1/2" not permitted.  
 Fig(s) \_\_\_\_\_

## 10. CHARACTER OF LINES, NUMBERS, &amp; LETTERS. 37 CFR 1.84(l)

☒ Lines, numbers & letters not uniformly thick and well defined, clean, durable, and black (except for color drawings).  
 Fig(s) ALL

## 11. SHADING. 37 CFR 1.84(m)

☐ Solid black shading areas not permitted.  
 Fig(s) \_\_\_\_\_

☐ Shade lines, pale, rough and blurred. Fig(s) \_\_\_\_\_

## 12. NUMBERS, LETTERS, &amp; REFERENCE CHARACTERS. 37 CFR 1.84(p)

☐ Numbers and reference characters not plain and legible. 37 CFR 1.84(p)(1) Fig(s) \_\_\_\_\_  
☐ Numbers and reference characters not oriented in same direction as the view. 37 CFR 1.84(p)(1) Fig(s) \_\_\_\_\_  
☐ English alphabet not used. 37 CFR 1.84(p)(2)  
 Fig(s) \_\_\_\_\_

☒ Numbers, letters, and reference characters do not measure at least .32 cm. (1/8 inch) in height. 37 CFR 1.84(p)(3)  
 Fig(s) 2, 5, 10

## 13. LEAD LINES. 37 CFR 1.84(q)

☐ Lead lines cross each other. Fig(s) \_\_\_\_\_  
☐ Lead lines missing. Fig(s) \_\_\_\_\_

## 14. NUMBERING OF SHEETS OF DRAWINGS. 37 CFR 1.84(r)

☐ Sheets not numbered consecutively, and in Arabic numerals, beginning with number 1. Sheet(s) \_\_\_\_\_

## 15. NUMBER OF VIEWS. 37 CFR 1.84(u)

☐ Views not numbered consecutively, and in Arabic numerals, beginning with number 1. Fig(s) \_\_\_\_\_  
☐ View numbers not preceded by the abbreviation Fig.  
 Fig(s) \_\_\_\_\_

## 16. CORRECTIONS. 37 CFR 1.84(w)

☐ Corrections not made from prior PTO-948.  
 Fig(s) \_\_\_\_\_

## 17. DESIGN DRAWING. 37 CFR 1.152

☐ Surface shading shown not appropriate. Fig(s) \_\_\_\_\_  
☐ Solid black shading not used for color contrast.  
 Fig(s) \_\_\_\_\_

COMMENTS:

Nov. 2. 2000 3:42PM MARSHALL, O'TOOLE

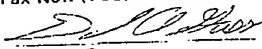
No. 5292 P. 2/2  
From: 0819

**PATENT**  
**28967/32863**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): Alitalo et al.  
Serial No: 08/510,133  
Filed: August 1, 1995  
Title: Receptor Ligand  
Group Art Unit: 1646  
Examiner: Christine Sacoud

Commissioner for Patents  
Washington, D.C. 20231

) I hereby certify that this paper is  
) being sent via facsimile to:  
) Commissioner for Patents,  
) Washington, D.C., 20231 on this  
) date: Date: November 2, 2000.  
) Fax No.: (703) 305-3014  
)   
) David A. Gass  
) Registration No. 38,153  
) Attorney for Applicants

**CHANGE OF ADDRESS**

Sir:

The undersigned is an attorney of record in this case. Please mail all correspondence in this case to the undersigned at the address below :

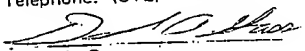
David A. Gass  
Marshall, O'Toole, Gerstein, Murray & Borun  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402

The attorney's phone number is (312) 474-6300.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

Dated: November 2, 2000

  
David A. Gass  
Registration No. 38,153

**OK to Enter**

B.3

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled RECEPTOR LIGAND, by inventor(s) Kari Alitalo and Vladimir Joukov

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above-identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

OK to Enter

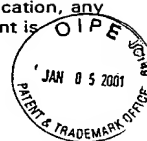
FULL NAME: Helsinki University Licensing, Ltd.  
ADDRESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland  
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that

such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.



NAME OF PERSON SIGNING: Edward A McDermott

TITLE IN ORGANIZATION: President

ADDRESS OF PERSON SIGNING: 1345 Avenue of the Americas, New York  
NY 10105

SIGNATURE: *Edward A. McDermott*

Date: March 2, 1997



**PATENT**

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov  
Serial or Patent No: 08/510,133  
Filed or Issued: August 1, 1995  
For: Receptor Ligand

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) -- SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ The owner of the small business concern identified below:
- ☒ An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Helsinki University Licensing, Ltd.

ADDRESS OF BUSINESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention, entitled Receptor Ligand, by inventor(s) Kari Alitalo and Vladimir Jovkov,

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
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Washington, D.C. 20231

APPLICATION NUMBER	08/01 FILING DATE	8/1/01	FIRST NAMED APPLICANT	K ATTORNEY DOCKET NO./328
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7542/0130

FRANK S. DIGIGLIO  
SCULLY SCOTT MURPHY & PRESSER  
400 GARDEN CITY PLAZA  
GARDEN CITY NY 11530

SAO EXAMINER	
436	
ART/JUNIT 7	PAPER NUMBER

01/30/01

DATE MAILED:

### Response to Rule 312 Communication

- ☐ The petition filed on \_\_\_\_\_ under 37 CFR 1.312(b) is granted. The paper has been forwarded to the examiner for consideration on the merits.

\_\_\_\_\_  
\_\_\_\_\_  
Director,  
Patent Examining Group \_\_\_\_\_

- ☒ The amendment filed on 1/3/01 under 37 CFR 1.312 has been considered, and has been:
- ☒ entered.
  - ☐ entered as directed to matters of form not affecting the scope of the invention (Order 3311).
  - ☐ disapproved. See explanation below.
  - ☐ entered in part. See explanation below.

*Kenna Scott*  
Publishing Division

The remaining amendments to the specification merely conform the specification to the formal drawings submitted concurrently herewith. Figures 2, 6, 9 and 10 were prepared on multiple sheets and/or renumbered in order to comply with the Draftsman's requirements. The specification has been amended to reflect the fact that these figures will be multiple pages in the issued patent.

These amendment add no new matter and do not raise any new patentability issues that would require any substantive examination by the Examiner.

In view of the foregoing, the applicant respectfully requests the granting of the amendment after allowance.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By: 

David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

January 3, 2001

the allowed claims. The Patent Office is authorized to charge any fee associated with this amendment to Deposit Account No. 13-2855.

The amendment to page 1 amounts to a cancellation of a priority claim to an application that was filed in November, 1994. The sole purpose behind cancellation of the priority claim is to maximize patent term of the eventual patent, because it is the Applicants' understanding of current law that the term of this patent will be measured from the earliest claimed priority date. The priority claim cancellation is not intended as an admission of whether or not the claimed invention would be entitled to priority, if the priority claim to the November, 1994 application were maintained. The Applicants reserve the right to maintain the same priority claim for subject matter that may be pursued in related applications, such as continuations, continuations-in-part, divisional applications, reissue applications, or the like. It is the Applicants' understanding from prosecution that the subject matter of the allowed claims has been deemed patentably distinct from any subject matter disclosed in art of record, including subject matter disclosed in two U.S. patents issued to Human Genome Sciences (Hu et al., U.S. Patent Nos. 5,932,540 and 5,935,820) that were considered by the Examiner. (One of these patents was cited by the Examiner as a reference under §102(e) and distinguished by the Applicants. See Amendment dated July 24, 2000, at pages 14-18.) Thus, the presence or absence of the priority claim raises no patentability issues.<sup>1</sup>

---

<sup>1</sup> The November, 1994 patent application has issued as U.S. Patent No. 5,776,755. The '755 patent is not prior art under §102(e) because, to the extent the '755 patent discloses or suggests the present invention, the relevant disclosure is a disclosure of the present inventors' own work. Because the relevant portions of the '755 patent constitute the inventor's own work, the relevant filing date of the '755 patent was not "before the invention thereof by the applicant" as required by §102(e). (It is impossible to disclose the inventors' own work before the inventors invented it.)

At page 7, line 25, please delete "Figure 5 shows" and insert --Figure 5A-5C show--.

At page 7, line 28, please delete "Figure 6 shows" and insert --Figures 6A and 6B show--.

At page 8, line 6, please delete "Figure 9 shows" and insert --Figures 9A through 9C show--.

At page 8, line 8, please delete "Figure 10 shows" and insert --Figures 10A and 10B show--.

At page 9, line 21, please delete "Figure 2" and insert --Figures 2A and 2B--.

At page 18, line 16, please delete "Figure 6" and insert --Figures 6A and 6B--.

At page 24, line 30, please delete "Figure 9" and insert --Figures 9A through 9C--.

At page 25, line 6, please delete "Figure 10" and insert --Figures 10A and 10B--.

At page 25, line 11, please delete "Fig. 9" and insert --Figures 9A through 9C--.

At page 26, line 7, please delete "Fig. 9" and insert --Figures 9A through 9C--.

At page 26, line 24, please delete "Fig. 10" and insert --Figure 10A--.

#### REMARKS

Applicants request entry of the foregoing amendments, which relate solely to formal matters. These amendments are being presented prior to or concurrently with payment of the issue fee as required by Rule 312. The amendments do not affect the scope or content of



the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME: Ludwig Institute for Cancer Research  
ADDRESS: 1345 Avenue of the Americas, New York, NY 10105  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☒ NONPROFIT ORGANIZATION

NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Heikki Lampi

TITLE OF PERSON OTHER THAN OWNER: President

ADDRESS OF PERSON SIGNING: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

SIGNATURE: \_\_\_\_\_

Date

27. Feb. 1997



01-05-01

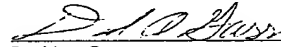
B

PATENT

Attorney Docket No.: 28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.	)	I hereby certify that this paper is being
Serial No: 08/510,133	)	deposited with the United States Postal
Filed: August 1, 1995	)	Service, in an envelope addressed to the:
Title: F1t4 LIGAND AND METHODS	)	Commissioner for Patents, Box Issue
OF USE	)	Fee, Washington, D.C. 20231, utilizing
	)	the "Express Mail Post Office" under
Allowed: October 3, 2000	)	Mailing Label No. EM578442453US on
	)	this date:
Batch No.: T33	)	
Group Art Unit: 1647	)	January 3, 2001
Examiner: C. Saoud	)	

  
David A. Gass

AMENDMENT AFTER ALLOWANCE PURSUANT 37 C.F.R. § 1.312

Commissioner for Patents  
Box Issue Fee  
Washington, D.C. 20231

Dear Sir:

Please amend this application as follows:

**OK to Enter**

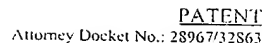
AMENDMENTS

In the Specification:

At page 1, line 2, please delete the following priority claim, which had been introduced by way of a Preliminary Amendment dated August 12, 1996:

"This application is a continuation-in-part of U.S. Patent Application Serial No. 08/340,011, filed November 14, 1994."

At page 7, line 19, please delete "Figure 2 shows" and insert --Figures 2A and 2B show--.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/510.133

Filed: August 1, 1995

Title: Flt4 LIGAND AND  
METHODS OF USE

Allowed: October 3, 2000

Batch No.: T33

Group Art Unit: 1647

Examiner: C. Saoud

) I hereby certify that this paper is being  
) deposited with the United States Postal  
) Service, in an envelope addressed to  
) the: Commissioner for Patents, Box  
) Issue Fee, Washington, D.C. 20231,  
) utilizing the "Express Mail Post  
) Office" under Mailing Label No.  
) EM57844255US on this date:

January 3, 2001

David A. Gass

## TRANSMITTAL OF FORMAL DRAWINGS

Commissioner for Patents  
Box Issue Fee  
Washington, D.C. 20231

Attention: Official Draftsman

Sir:

In response to the requirement made in the notice of allowability dated October 3, 2000, the applicants, through their undersigned attorney, submit herewith 16 sheets of formal drawings (Figures 1-12).

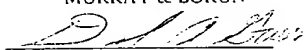
Because Figures 2, 9 and 10 are now each depicted on multiple sheets, and Figure 6 depicted as Figure 6A and Figure 6B on one sheet, the formal drawings are accompanied by an amendment after allowance which updates cross-references to the Figures.

This application was allowed October 3, 2000. The issue fee is being transmitted today under separate cover. Acceptance of the submitted formal drawings is solicited.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By

  
David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

January 3, 2001



6221839

1 / 16

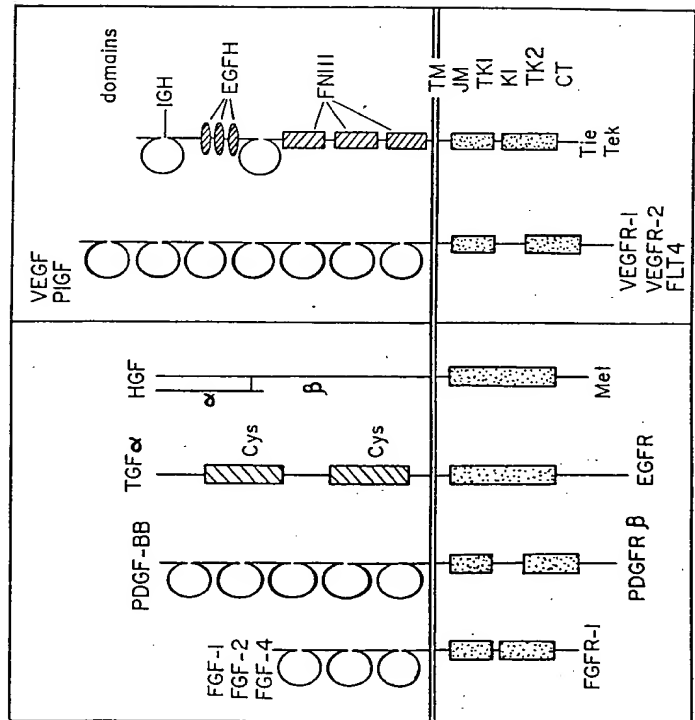


FIGURE 1

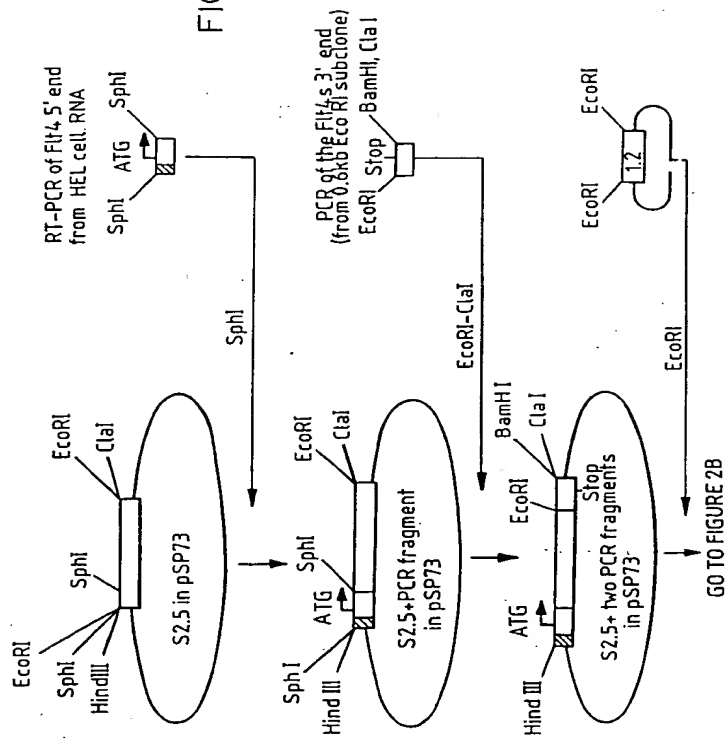
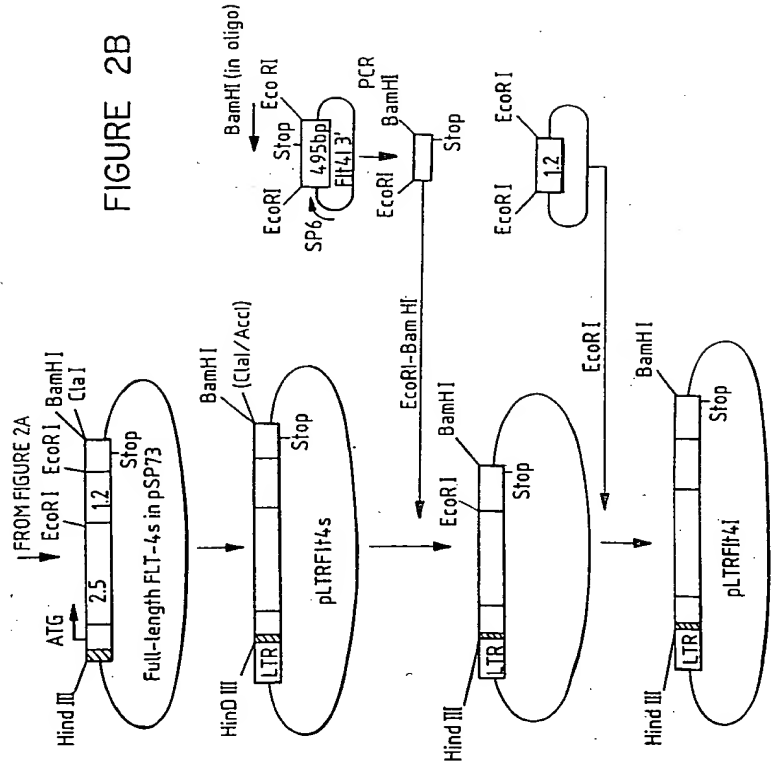


FIGURE 2A

FIGURE 2B



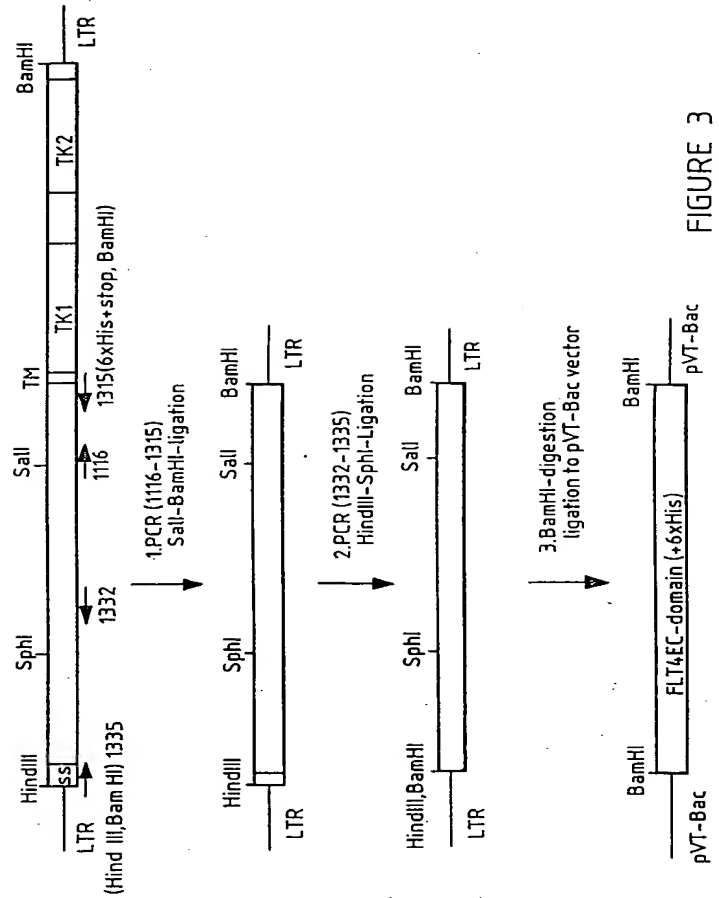


FIGURE 3

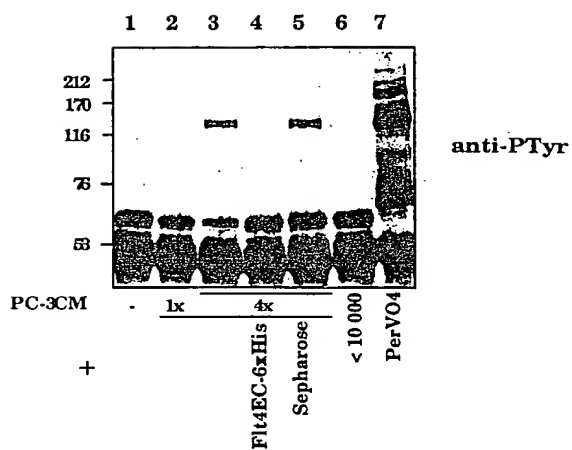
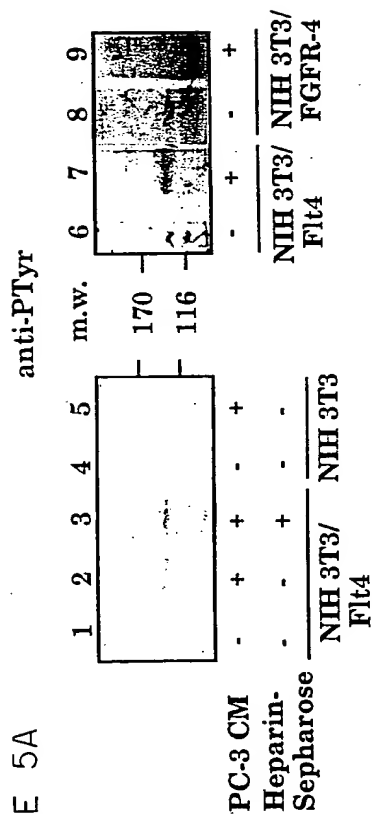
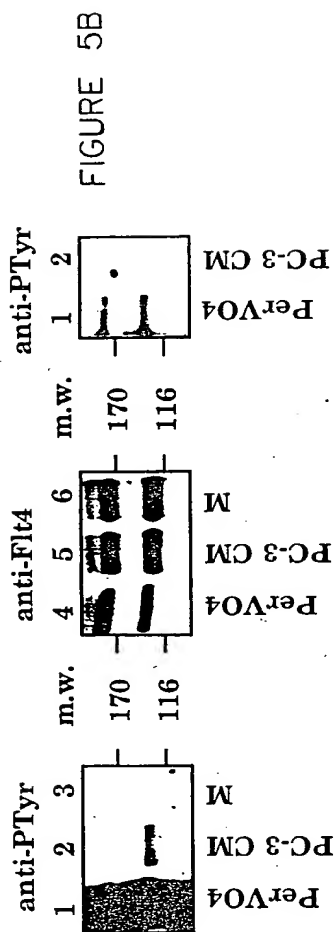


FIGURE 4



7 / 16

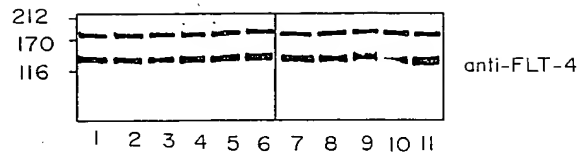


FIGURE 6A

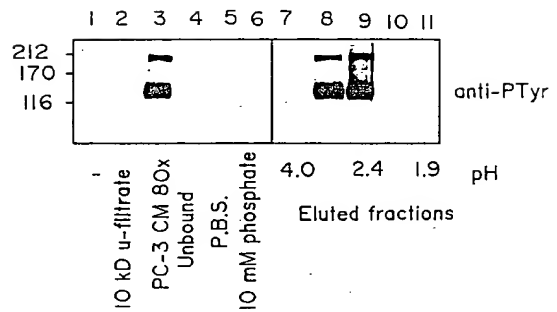


FIGURE 6B

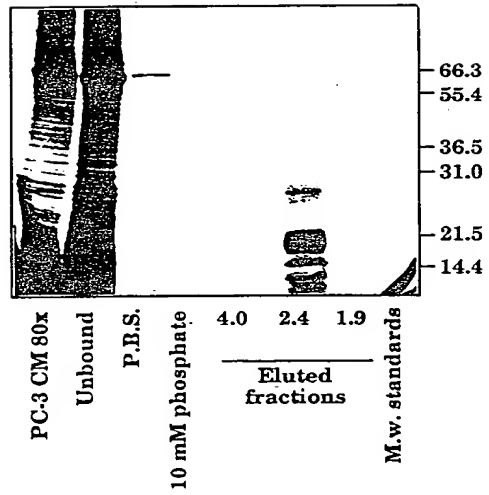


FIGURE 7



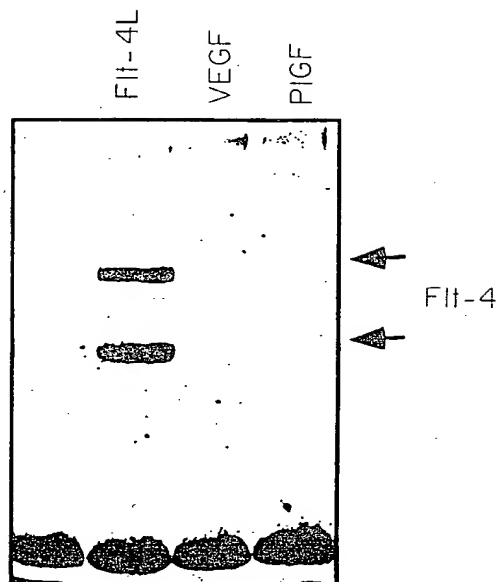


FIGURE 8

MetThrValLeuTyrProGluTyr  
 GAGCAGTTACGGTCTGTCTCCAGTGTAGATGAACCTCATGTACTGTACTCTACCCAGAATAT  
 10 30 50

TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla  
 TGGAAAATGTACAAGTGTCAAGTCTAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC  
 70 90 110

AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaHisTyrAsnThrGlu  
 AACCTCAACTCAAGGACAGAGAGACTATAAAATTTGCTGCAGCACATTAATAACAGAG  
 130 150 170

IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys  
 ATCTTGAAAAGTATTGATATAATGAGTGGAGAGAAAGACTCAATGCATGCCACGGGAGGTGTGT  
 190 210 230

IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProCysVal  
 ATAGATGTGGGAAGGAGTTTGGAGTCGCGACAAACACCTTCTTTAAACCTCCATGTGTG  
 250 270 290

SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer  
 TCCGTCTACAGATGTGGGGTGTGCTGCAATAGTAGGGGGCTGCAGTGCATGAACACCCAGC  
 310 330 350

FIGURE 9A

ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys  
 ACAGCTACCTCAGCAAGACGTTATTGAAATTACAGTGCCTCTCTCAAGGCCCAAA  
 370 390 410  
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal  
 CCAGTAACAATCAGTTTTTGCCAATCACACTTCCTGCCGATGCATGCTCTAACTGGATGTT  
 430 450 470  
 TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln  
 TACAGACAAGTTCATTCCCATTTATAGACGTTCCCTGCCAGCAACACTACACAGTGTCTAG  
 490 510 530  
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu  
 GCAGCGAACAAGACCTGCCCCACCAATTACATGTGGAATAATCACATCTCAGATGCCTG  
 550 570 590  
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis  
 GCTCAGGAAGATTTTATGTTTTTCCTCGGATGCTGGAGATGACTCAACAGATGGATTCCAT  
 610 630 650  
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla  
 GACATCTGTGGACCAACAAGGAGCTGGATGAAGACCTGTCAGTGTGTCTGCAGAGCG  
 670 690 710

FIGURE 9B

GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys  
GGGCTTCGGCCTGCCAGCTGTGGACCCCAAGAACTAGACAGAACTCATGCCAGTGT  
730 750 770

ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn  
GTCTGTAAAAACAAACTCTTCCCCAGCCCAATGTGGGGCCCAACCGAGAAATTTGATGAAAAAC  
790 810 830

ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys  
ACATGCCAGTGTGTATGTAAAGAACTGCCCCAGAAATCAACCCCTAAATCCTGGAAAAA  
850 870 890

CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysLysPheHis  
TGTGCCGTGTGATGTACAGAAAGTCCACAGAAATGCTTGTAAAGGAAAGAGTCCAC  
910 930 950

HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro  
CACCAAAATGCAGCTGTACAGACGGCCATGTACGAACCGCCAGAGGCTGTGAGCCA  
970 990 1010

GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln  
GGATTTTCATATAGTGAAGAGTGTGTCGTGTGTCCTTCATATTTGAAAGAACCCACAA  
1030 1050 1070

MetSerEnd  
ATGAGCTAAGATGTACTGTTTCCAGTTCATCGATTTTCTATTATGGAAGAACTGTGTTG  
1090 1110 1130

1					50
PDGF-A	.MRTWACLLL	LGCGYLAHAL	ABEAEIPREL	IERLARSQIH	SIFDLQRLLE
PDGF-B	MNRCAWAL	LFLSLCCYLRLVS	AEGDPIPEEL	YEMLSHDHSR	SFDDLQRLHH
PLGF	.....	.....	.....MP	VMRLFPFCFLQ	LLAGLAL...
VEGF	.....	.....	.....	MNLLSWVH	WSLALLLYLH
FLT4-L	.....	.....	.....	MTVLYPEYWK	MYKCQLRKGG
51					100
PDGF-A	IDSVGAEDAL	ETSLRAHGS	AINHVPEKRP	VPIRRKRSL	.....EEAIP
PDGF-B	GDP.GEEDGA	ELDLNMTRSH	SGGELES...	LARGRRSLG	SLTIAEPAMI
PLGF	PAVPPQQWAL	SA.....	NGSSEVEV	P.FQEVWG..	.....R
VEGF	HAKWSQAAPM	AE.....	GGQNHHEV	K.FMDVYQ..	.....R
FLT4-L	WQHNREQANL	NSRTEETIKF	AAAHYNTAIL	KSIDNEW..	.....K
101					150
PDGF-A	AVCKTRTVIY	EIPRSQVDPT	SANFLWPPC	VEVRCCHGCC	NTSSVKQPS
PDGF-B	AECKTRTEVF	EISRRLLDRT	NANFLVWPPC	VEVQCSGCC	NNRNVCQRPT
PLGF	SYCPALERIV	DVSEY..PS	EVEHMFSPSC	VSLIRCHGCC	GDENLHVVPV
VEGF	SYCHPIETLV	DIFQY..PD	EIEYIFKPPC	VPLMRQGGCC	NDEGLECVPT
FLT4-L	TQCMPREVCI	DVGKEF..GV	ATNTFFKPPC	VSVYRQGGCC	NSEGLOQNT
151					200
PDGF-A	RVHHSVKVA	KVEYVRKKPK	LKEVQVRLEE	FILEQAC....	AT.....
PDGF-B	QVQLRPVQVR	KIEIVRKKPI	EKKATVTLED	HLACKC....	ETVAARPVT
PLGF	ETANVTMQLL	KIRSG..DRP	SYVELTFSQ	HVRCECRPLR	EKKMPERC..
VEGF	EESNITMQIM	RIKPH..QQQ	HIGEMSFLQ	HNKCECRPKK	DRARQENP..
FLT4-L	STSVLSKTLF	EITVPLSQGP	KPVTISFAN	ETSQMSKL	DVYRQVHSII

FIGURE 10A

201	PDGF-A	..SNLNPDR	EEETDVR...	.....	.....	250
	PDGF-B	RSPGSGQQR	AKTPQTRVTI	RTVVRPPK	GKHKFKHTH DKTALKETLG	
	PlGF	.....	GDAVRR...	.....	.....	
	VEGF	.....	CGPCSEKHK	LFVQDPQCK	CCKNTDSRC KARQLELNER	
	FLT4-L	RRSLPATLPQ	QQAANKTCPT	NYMNNHICR	CLAQEDFMFS SDAGDDSTDG	300
251						
	PDGF-A	.....	.....	.....	.....	
	PDGF-B	A.....	.....	.....	.....	
	PlGF	.....	.....	.....	.....	
	VEGF	TCRCDKPRR.	.....	.....	.....	
	FLT4-L	FHDICGPNKE	LDEETQCVC	RAGLRPASCG	PHKELDRNSC QCVCNKLFP	350
301						
	PDGF-A	.....	.....	.....	.....	
	PDGF-B	.....	.....	.....	.....	
	PlGF	.....	.....	.....	.....	
	VEGF	.....	.....	.....	.....	
	FLT4-L	SQCGANREFD	ENTQCVCCKR	TCPRNQPLNP	GKCACECTES POKCLLKGGK	395
351						
	PDGF-A	.....	.....	.....	.....	
	PDGF-B	.....	.....	.....	.....	
	PlGF	.....	.....	.....	.....	
	VEGF	.....	.....	.....	.....	
	FLT4-L	FHHQTCSCYR	RPCTNROKAC	EPGFSYSEEV	CRCVPSYWKR PQMS	

FIGURE 10B

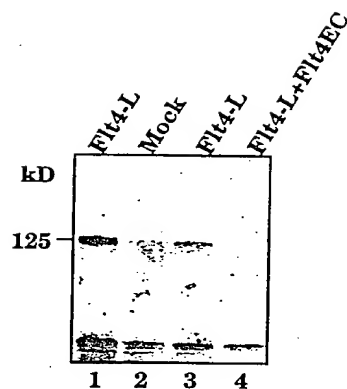


FIGURE II

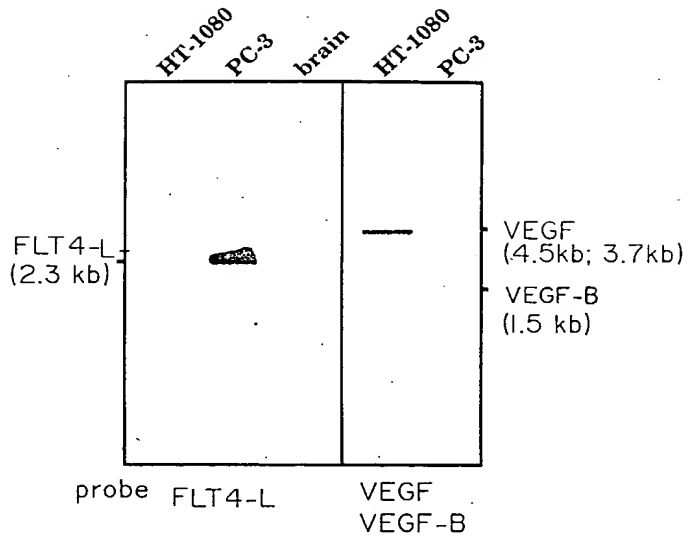


FIGURE 12





US006221839B1

**(12) United States Patent**  
**Alitalo et al.****(10) Patent No.: US 6,221,839 B1**  
**(45) Date of Patent: Apr. 24, 2001****(54) FLT4 LIGAND AND METHODS OF USE****(75) Inventors:** Kari Alitalo, Espoo; Vladimir Joukov, Helsinki, both of (FI)**(73) Assignee:** Helsinki University Licensing Ltd. Oy, Helsinki (FI); Ludwig Institute for Cancer Research, New York**(\*) Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.**(21) Appl. No.:** 08/510,133**(22) Filed:** Aug. 1, 1995**(51) Int. Cl.:** A61K 38/18; C07K 14/475**(52) U.S. Cl.:** 514/12; 514/2; 530/399**(58) Field of Search:** 530/399; 514/2; 514/21**References Cited****U.S. PATENT DOCUMENTS**

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(List continued on next page.)

**Primary Examiner**—Christine Saoud  
(74) **Attorney, Agent, or Firm**—Marshall, O'Toole, Gerstein, Murray & Borun

**ABSTRACT**

**(57)** Provided are ligands for the receptor tyrosine kinase, Flt4. Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.

29 Claims, 16 Drawing Sheets

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Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro  
340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys  
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr  
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser  
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro  
405 410 415

Gln Met Ser

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala  
 35 40 45  
 Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser  
 50 55 60  
 Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met  
 65 70 75 80  
 Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln  
 85 90 95  
 Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala  
 100 105 110  
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys  
 115 120 125  
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe  
 130 135 140  
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr  
 145 150 155 160  
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr  
 165 170 175  
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu  
 180 185 190  
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser  
 195 200 205  
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile  
 210 215 220  
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn  
 225 230 235 240  
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys  
 245 250 255  
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser  
 260 265 270  
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu  
 275 280 285  
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys  
 290 295 300  
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys  
 305 310 315 320  
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu  
 325 330 335

CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT GTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA GAA GTG TGT CGT TGT GTC CCT TCA TAT TGS AAA AGA CCA CAA ATG Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met 405 410 415	1605
AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT TTTCTATTAT GGAAAACCTGT Ser	1658
GTGCCCACAG TAGAACTGTC TGTGAACAGA GAGACCCCTTG TGGGTCCATG CTAACAAAGA	1718
CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAATGGACT GGAGCTCATC	1778
TGCAAAAGGC CTCTTGTAAG GACTGGTTTT CTGCCAATGA CCAACACGCC AAGATTTTCC	1838
TCTTGTAATT TCTTTAAAG AATGACTATA TAATTTATIT CCACTAAAAA TATTGTTTCT	1898
GCATTCATIT TTATAGCAAC AACAAATTGGT AAAACTCACT GTGATCAATA TTTTATATC	1958
ATGCAAAATA TGITTAAAAAT AAAATGAAAA TTGTATTAT	1997

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 419 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala	
1 5 10 15	
Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe	
20 25 30	

CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr	
100 105 110	
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
115 120 125 130	
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Cys Val Ser Val Tyr Arg Cys	
150 155 160	
GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG	885
Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr	
165 170 175	
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA	933
Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln	
180 185 190	
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA	981
Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg	
195 200 205 210	
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA	1029
Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg	
215 220 225	
CGT TCC CTG CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC	1077
Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr	
230 235 240	
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT	1125
Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala	
245 250 255	
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT	1173
Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp	
260 265 270	
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC	1221
Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr	
275 280 285 290	
TGT CAG TGT GTC TGC AGA GCG GGG CIT CGG CCT GCC AGC TGT GGA CCC	1269
Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro	
295 300 305	
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA	1317
His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys	
310 315 320	

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1997 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
CCCGCCCCGC CTCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGGCGTC CTCCTTCGCC      60
CTCGCTTCAC CTCGCGGGCT CCGAATGCGG GGAGCTCGGA TGTCGGTTT CCTGTGAGGC      120
TTTTCACCTGA CACCCGCGGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAAGTTG      180
GGAACGCGGA GCCCGGACCG CGCTCCCGCC GCCTCCGGCT CGCCAGGGG GGGTCGCCGG      240
GAGGAGCCCG GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC      300
CCACCCCTGC CCCCGCCAGC GGACCGGTCC CCCACCCCGG GTCCTTCCAC C ATG CAC      357
                                         Met His
                                         1

TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG      405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu
                    5                      10                      15

CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCC GCC GCC TTC GAG TCC      453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser
    20                      25                      30

GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT      501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala
    35                      40                      45                      50

TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA      549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln His Arg Ser Val Ser Ser Val
                    55                      60                      65

GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG      597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
                    70                      75                      80

TGT CAG CTA AGG AAA GGA GGC TGG CAA CAT AAC AGA GAA CAG GCC AAC      645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn
                    85                      90                      95
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the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the application or any patent issued thereon.

July 17, 1998  
Date

Kari Alitalo  
Kari Alitalo



basis that such an amendment "introduces new matter into the disclosure." The Patent Office's basis for this allegation was as follows:

The specification discloses that the Flt4-L clone has an approximately 2.1 kb insert and has been deposited as ATCC Deposit No. 97231 (pp. 28-29). Applicant has not stated or shown the relationship between the 2.1 kb insert and the 1997 bp cDNA sequenced and presented as SEQ ID NO: 44. Thus, it is not clear whether the 2.1 kb insert has the sequence of SEQ ID NO: 44. If the 1997 bp insert is the same as that of the 2.1 kb insert, this aspect of the rejection could be overcome by amending the sentence added in the amendment of 1 December 1997 to state that "the approximately 2.1 kb cDNA insert of the deposited plasmid pFLT4-L was sequenced and found to have a 1997 base pair nucleotide sequence as set forth in SEQ ID NO: 44."

(Office action dated March 24, 1998, at paragraph 10.)

3. I confirm that our laboratory sequenced the insert of the same plasmid that was designated pFLT4-L and that was deposited with the ATCC as ATCC Deposit No. 97231 and that is referred to at pages 28-29 of the patent application. The nucleotide sequence of the insert of this plasmid (ATCC Deposit No. 97231) includes the 1997 nucleotides of sequence set forth in SEQ ID NO: 44 as appended hereto and added to the patent application in the amendment dated November 26, 1997. The 419 residue amino acid sequence set forth in SEQ ID NO: 45 (as appended hereto and added to the patent application) is deduced from the sequence set forth in SEQ ID NO: 44.

4. The insert of plasmid pFLT4-L (ATCC Deposit No. 97231) contains additional (non-coding) sequence adjacent to the 1997 nucleotides of sequence set forth in SEQ ID NO: 44. The apparent size discrepancy between the approximately 2.1 kb size of the insert (as estimated by agarose gel electrophoresis analysis) and the 1997 nucleotides of sequence as set forth in SEQ ID NO: 44 is explained by the existence of this additional non-coding sequence in the plasmid insert.

#### Certification

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and

EXHIBIT

PATENT  
28967/33072

IN THE UNITED STATES  
PATENT AND TRADEMARK OFFICE

In re Application of: ) I hereby certify that this paper is being  
) deposited with the United States Postal  
Alitalo et al. ) Service as first class mail, postage  
) prepaid, in an envelope addressed to:  
Serial No.: 08/585,895 ) Assistant Commissioner for Patents  
) Washington, D.C. 20231, on this date:  
Filed: January 12, 1996 ) Dated: July 23, 1998  
)  
Title: RECEPTOR LIGAND )  
)  
Art Unit: 1646 )  
)  
Examiner: Saoud ) David A. Gass

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. KARI ALITALO

I, Kari Alitalo, do hereby declare and state as follows:

1. I am a co-inventor of the above-identified U.S. Patent Application (hereinafter "the patent application"). I am familiar with the Office action from the U.S. Patent and Trademark Office dated March 24, 1998, in the patent application. I am making this declaration to provide facts and evidence to the Patent Office that may be relevant to the issues and rejections raised in the Office action.

2. I understand that sequences identified as SEQ ID NOS: 44 and 45 were added to the patent application by an amendment dated November 26, 1997, and entered by the Patent Office on December 1, 1997. Copies of those two sequences are appended hereto. I understand that, at the time of the amendment, SEQ ID NOS: 44 and 45 were identified as a nucleotide sequence and a deduced amino acid sequence of a cDNA that was deposited with the American Type Culture Collection (ATCC) as plasmid pFLT4-L and that is cross-referenced in the patent application at pages 28-29. I understand that the Patent Office has objected to the amendment to introduce these two sequences into the patent application on the

amendment and the ATCC deposit are further corroborated by the attached copies of two Declaration under 37 C.F.R. §1.132 of Dr. Kari Alitalo that were filed for a related patent application, U.S. Serial No. 08/585,895. SEQ ID NOS: 34 and 35 of the substitute sequence listing for the present application (U.S. Serial No. 08/510,133) are identical to SEQ ID NOS: 44 and 45 of both Declarations. Paragraphs 4 & 5 of the Declaration filed November 26, 1997 and paragraphs 2-4 of the Declaration filed July 23, 1998 explain that complete sequencing of the cDNA insert that was deposited with the ATCC revealed a sequence that includes a 1997 base pair nucleotide sequence (SEQ ID NO: 34 of the substitute Sequence Listing field herewith) that encodes a 419 amino acid sequence (SEQ ID NO: 35 of the substitute Sequence Listing filed herewith). Thus, the present amendment is supported by the application as filed and does not introduce new matter. Entry of the amendment is respectfully requested.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, IL 60606-6402  
Telephone: (312) 474-6300

By: 

David A. Gass  
Registration No. 38,153

August 22, 2000

AMENDMENTS

Please amend the specification as follows:

At page 26, line 12, after "97231.", please insert --A 1997 base pair nucleotide sequence and the deduced amino acid sequence of the cDNA insert of this deposited plasmid is set forth in SEQ ID NOS: 34 and 35, respectively.--

Please delete pages 30-40 of the specification, which comprise the original Sequence Listing, and substitute therefor new pages 30-44, filed herewith, which constitute a substitute Sequence Listing. In view of this amendment, please renumber the pages of claims and abstract beginning with "45" (to preserve consecutive page numbering).

REMARKS

The present amendment modifies the application by introducing a 1997 bp DNA and deduced amino acid sequence corresponding to a Budapest treaty (ATCC) cDNA deposit that is referred to at page 26 and elsewhere in the application. The amendment to introduce these sequences will benefit the interested public by producing additional relevant information about the invention in the issued patent. None of the pending claims recites SEQ ID NOS: 34 or 35.

I hereby state that the content of the paper and computer-readable forms of the substitute Sequence Listing submitted herewith, for entry as part of the above-identified application, are the same as each other and do not introduce new matter into the disclosure of the application. All of the sequence information embodied in the substitute Sequence Listing filed herewith finds support in the application as originally filed, as explained below:

SEQ ID NOS: 1-33 of the original and substitute Sequence Listings are identical. Therefore, no new matter has been introduced in these sequences.

SEQ ID NOS: 34-35 of the substitute Sequence Listing depict a 1997 base pair nucleotide sequence and a deduced amino acid sequence, respectively, of a cDNA that was deposited with the ATCC and cross-referenced at p. 26 of the patent application as filed. These sequences are inherent properties of the deposited plasmid and thus find support in the deposited plasmid itself. See *Kennecott Corp. v. Kyocera International Inc.* 5 U.S.P.Q.2d 1194 (Fed. Cir. 1987) (The express description of an inherent property is not new matter and can be added to a specification with effect as of the original filing date); *In re Lundak*, 227 U.S.P.Q. 90 (Fed. Cir. 1985). The correlation between the sequences introduced by this

1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.  
 Has not been established.

2. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	29	MINUS	23	= 6	X 9=	\$54.00	X18=	\$
INDEP.	4	MINUS	5	= 0	X39=	\$0	X78=	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+130=	\$	+260=	\$
TOTAL ADDITIONAL FEE						\$54.00	OR	\$

3. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: **\$54.00**  
☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
 A copy of this Transmittal is enclosed.

4. **Deposit Account and Refund Authorization**

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.  
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, OTOOLE, GERSTEIN,  
 MURRAY & BORUN  
 6300 Sears Tower  
 233 South Wacker Drive  
 Chicago, Illinois 60606-6402  
 (312) 474-6300

By: 

David A. Gass  
 Reg. No: 38,153

July 24, 2000

RECEIVED

SEP 1 1995

WJD

TECH CENTER 1600/2900 PATENT  
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Alitalo et al.	)	For: RECEPTOR LIGAND
Serial No. 08/510,133	)	Art Unit: 1646
Filed: August 1, 1995	)	Examiner: Saoud. C.

RECEIVED

TECH CENTER 1600/2900 PATENT

AMENDMENT AND STATEMENT PURSUANT TO 37 C.F.R. §1.825

Group 1600  
Crystal Mall I  
7<sup>th</sup> Floor Reception Desk  
Washington, D.C. 20231

FILED  
1/15/95

Sir:

The Applicants request entry of the following amendment in the above-identified patent application prior to issuance of the next action on the merits.



GAU 1646

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	) Title: RECEPTOR LIGAND
Alitalo et al.	)
Serial No: 08/510,133	)
	) Group Art Unit: 1646
Filed: August 1, 1995	) Examiner: Christine Saoud

AMENDMENT TRANSMITTAL

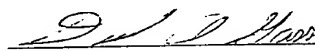
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment for the above application.

CERTIFICATE OF MAILING (37 CFR 1.8)

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 24, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

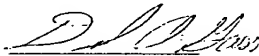
**IV: Conclusion**

The Applicants respectfully request entry of the foregoing amendments and allowance of all of the pending claims in view of the foregoing remarks.

Respectfully submitted,

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Telephone: (312) 474-6300

Dated: July 24, 2000



David A. Gass  
Registration No. 38,153



- approximately 23 kD (see, e.g., claim 35 and claims dependent therefrom);
- (2) A polypeptide comprising a specified amino terminus defined with respect to SEQ ID NO: 13 (see, e.g., claims 45 and claims dependent therefrom, and claim 43);
  - (3) A polypeptide that binds Flt4, and comprises a continuous portion of SEQ ID NO: 33, said portion consisting of a portion within amino acids 1-180 of SEQ ID NO: 33 (see, e.g., claims 29 and claims dependent therefrom and claim 42); and
  - (4) A polypeptide of about 23 kD that is purifiable from PC-3-conditioned medium using Flt4 affinity procedures, as recited in claim 51 and claims dependent therefrom;<sup>7</sup>

The Patent Office has not pointed to any description or suggestion in Hu et al. of any of these features, and in fact, Hu et al. neither describes nor suggests such features.

Because all of the claims recite features that are neither disclosed nor suggested by Hu et al., the rejection under §102(e) must be withdrawn.

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<sup>7</sup> It is clear from the Application (e.g., pp. 18-19 and Figure 7) that the polypeptide purifiable from PC3 medium is not prepro-VEGF-C, but rather, e.g., a 23 kD form having an amino-terminal sequence corresponding to XEETIKFAAAHYNTEILK.

December 24, 1997, more than two years after the filing date of the present application, and after the publication of a PCT application based on the present application (See WO 97/05250, published February 13, 1997), and after the publication of the present inventors own work in prominent scientific journals that would have come to the attention of Hu et al. (See, e.g., Joukov et al., "A Novel Vascular Endothelial Growth Factor, VEGF-C, Is a Ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) Receptor Tyrosine Kinases," *EMBO J.*, 15(2): 290-298 (1996); and Joukov et al., "Proteolytic Processing regulates receptor specificity and activity of VEGF-C," *EMBO J.*, 16(13): 3898-3911 (1997).) Still more of the inventors' work was published in 1997-1999, during the pendency of the Hu et al. application, when Hu et al. had the opportunity to amend their claims. The relevant inquiry under §102(e) is the inquiry of what was "described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent." This inquiry requires the Patent Office to ignore what was claimed in the Hu et al. patent, which may have been tainted by knowledge of the present invention, as explained above. The relevant inquiry must focus only on what was described in those Hu et al. priority applications that have a filing date that could have preceded the invention date of the applicants.<sup>6</sup> See, e.g., *In re Benno*, 226 USPQ 683, 686 (Fed. Cir. 1985) ("The scope of a patent's claims determines what infringes the patent; it is no measure of what it discloses. A patent discloses only that which it describes....")

Moreover, the Patent Office has apparently ignored the axiom that anticipation of a claim under §102 can be found only if the prior art discloses every element of the claim. See, e.g., *In re King*, 801 F.2d 1324, 1326 (Fed. Cir. 1986). The claims (as pending and amended herein) recite features that are neither disclosed nor suggested by Hu et al., including the following features:

- (1) A polypeptide that binds Flt4, comprises a portion of the SEQ ID NO: 33 amino acid sequence, and comprises a molecular weight of

---

<sup>6</sup> The Applicants reserve the right to dispute whether Hu et al. qualifies as a §102(e) reference, on the grounds that Hu et al. is not a patent granted on an application filed before the invention thereof by the applicant.

etc.) for allelic variants and the like, using, e.g., conventional hybridization procedures. Assuming that one uses this polynucleotide screening-based approach to identify polynucleotides that potentially encode alternative human alleles, the application also teaches Flt4 binding and activity assays to confirm that any novel sequences that one obtains, encodes, and expresses will satisfy the functional limitations of the claims.

For all of these reasons, the claims in the application are commensurate in scope with the subject matter enabled by the specification, and the rejection under §112, first paragraph, for lack of enablement should be withdrawn.

**V. The rejection of claims 1-2, 8-9, 12-17, and 19-28 under 35 USC §102(e) as being anticipated by Hu et al. was improper, and should be withdrawn.**

The Patent Office rejected claims 1-2, 8-9, 12-17, and 19-28 under 35 USC §102(e), alleging that the subject matter of these claims was anticipated by Hu et al., U.S. Patent No. 5,932,540:

Hu et al. disclose a polypeptide, SEQ ID NO:2, which is capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation of mammalian cells expressing Flt4 receptor tyrosine kinase (see claims 1-60). Therefore, the instant claims are anticipated by the prior art.

With regard to claims 19, 21, and 28 which include a detectable label, Hu et al. disclose the polypeptide linked to a detectable label at column 17, lines 60-65, thus meeting this limitation.

(Office Action at pp. 9-10.)

The Applicants respectfully traverse.

At the outset, the Applicants dispute the Patent Office's characterization of Hu et al., because Hu et al. neither discloses nor suggests that any polypeptide binds Flt4. In fact, Hu et al. makes no mention of the Flt4 receptor whatsoever.

The Applicants also dispute the Patent Office's implication that the scope or wording of *the claims* of the Hu et al. patent have any relevance to whether Hu et al. is anticipatory under §102(e). The Hu et al. application was filed on

17, 20-22, 25, and 28). As set forth below, the rejection of these claims (and their dependent claims) also should be withdrawn.

As explained in detail in the arguments relating to written description, claim 45 recites "human" and claim 51 recites purifiable from a human cell line. Claim 45 also recites a partial amino acid sequence to further identify the human polypeptide. The claims recite that the polypeptides have Flt4 binding activity, and the specification demonstrates that Flt4 affinity chromatography can be used successfully to isolate the claimed polypeptides.

The Patent Office urges that "The claims must recite sufficient structural elements to provide the recited functions, and one of ordinary skill in the art would not reasonably expect any 23 kD protein or protein which comprises SEQ ID NO:13 to bind to Flt4." The Applicants dispute that §112, first paragraph, contains any requirement of this sort. As explained above, 35 USC §112, first paragraph, sets forth minimum requirements for *the specification*, not for the claims. The purpose of patent claims is to particularly point out and distinctly claim the subject matter of the invention (§112, second paragraph), in a way that apprises the public of what is within the scope of the invention and what is not. It is not questioned that the pending claims satisfy this requirement.

If one makes the inquiry under §112, first paragraph, of whether *the specification* is enabling for the full scope of the claims, the answer is clearly affirmative. The Patent Office's rejection is clearly focused on the "how to make" aspect of the enablement requirement, and the present application teaches those skilled in the art several methodologies to make the subject matter of the invention, commensurate with the scope of claims 45 and 51 and dependent claims. For example, the patent application teaches that one can use affinity purification procedures to isolate the claimed polypeptide from cell sources. (The application also enables the production of antibodies to the receptor ligand that can be used for affinity purification procedures.) The application also teaches a human polynucleotide sequence that one can use to make polypeptides of the invention using the entire breadth of recombinant technologies known in the art. Additionally, because the application teaches a cDNA sequence, it enables one of ordinary skill in the art to screen any human source (human cell lines, human biological samples,

IV. The Patent Office's rejection of claims 1, 12-17, 19, 21-23, 25, and 28 under 35 U.S.C. §112, first paragraph, for lack of enabling disclosure should be withdrawn.

In paragraph 7 of the Office action, the Patent Office rejected claims 1, 12-17, 19, 21-23, 25, and 28 under 35 U.S.C. §112, first paragraph, alleging that "the specification, while being enabling for polypeptides comprising a contiguous portion of SEQ ID NO:33 which specifically bind to Flt4 receptor tyrosine kinase, does not reasonably provide enablement for any polypeptide that specifically binds to Flt4 receptor tyrosine kinase, or for those polypeptides that have a molecular weight of 23 kD and bind, or for those polypeptides which comprise amino acid sequence SEQ ID NO:13 and bind. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims." The Applicants respectfully traverse.

A. The rejection of claims 1, 12, 14-15, 19, and 23 has been rendered moot.

The scope of the rejection also has been narrowed by the amendments set forth above. Specifically, the Applicants have canceled rejected claims 1 and 23.<sup>5</sup> The new claims which correspond to several of the other rejected claims (e.g., claims 12, 14, 15, 19) have been amended to depend (directly or indirectly) from claims 29 and 35 (which are analogous to claims 8 and 9, which the Patent Office had acknowledged to be enabled by the specification). The rejection of claims 12, 14, 15, and 19 should therefore be withdrawn.

B. The rejection of claims 13, 16, 17, 21-22, 25, and 28 should be withdrawn.

Claims 45 and 51 (which correspond with rejected claims 16 and 17) are the two independent claims of the remaining claims that stand rejected (13, 16,

<sup>5</sup> The Patent Office's main allegation in support of its rejection is that "The broadest claim only requires the polypeptide to be capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The additional limitations of the dependent claims fail to provide the structure which is required for this receptor binding or for receptor activation (an additional functional limitation)." (Office action at p. 3.) As explained above, claim 1, the claim that required only binding, has been canceled without prejudice.

The claims lack structural limitations (i.e. claims 1, 14, 17, 19, 20, and 21 recite no structure at all other than a polypeptide) to provide the function of encoding a polypeptide which binds to the Flt4 receptor tyrosine kinase. Some of the claims include a molecular weight, however, this is not sufficient for providing the required function and some of the claims recite a portion of SEQ ID NO:13, however, this amino acid sequence is still not sufficient for providing the receptor binding activity required by the claims.

[T]he instant application fails to provide a written description of the species or the genus which are encompassed by the instant claims except for the polypeptide of SEQ ID NO:33. The specification does not provide a complete structure of those polypeptides which bind to the Flt4 receptor tyrosine kinase with high affinity. The claims also fail to recite other relevant identifying characteristics (physical and/or chemical and/or functional characteristics coupled with a known or disclosed correlation between function and structure) sufficient to describe the claimed invention in such full, clear, concise and exact terms that a skilled artisan would recognize applicant was in possession of the claimed invention.

(Office action at pp. 4-5.)

The Patent Office is reminded that 35 USC §112, *first paragraph*, sets forth minimum requirements for *the specification*, not for the claims. The purpose of patent claims is to particularly point out and distinctly claim the subject matter of the invention (§112, *second paragraph*), in a way that apprises the public of what is within the scope of the invention and what is not. The pending claims satisfy this requirement.<sup>4</sup>

For all of these reasons, the rejection for lack of written description should be withdrawn.

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<sup>4</sup> No paragraph of Section 112 requires claim limitations to provide function as suggested by the Examiner. This is aptly demonstrated by the facts that (1) the Patent Office issued patent claims to proteins prior to the revolution of recombinant DNA technology which provided sequencing capabilities; and (2) the Patent Office continues to issue countless claims to antibodies (which are proteins) that do not recite variable region amino acid sequence or otherwise characterize the antibody variable region, except in functional terms.

U.S.C. 112, first paragraph, it is necessary to understand what Applicant has possession of and what Applicant is claiming." (Office action at p. 3.) However, when performing this analysis, the Patent Office then focused on nucleic acids:

From the specification, it is clear that Applicant has possession of a nucleic acid molecule which encodes a protein which has the amino acid sequence of SEQ ID NO:33. This nucleic acid molecule has a nucleic acid sequence of SEQ ID NO:32 and is contained within plasmid pFLT4-L (ATCC deposit #97231).

(Id.)

This decision to focus on nucleic acids suggests a possible misapprehension of the application, and a misapplication of "nucleic acid reasoning" to a polypeptide invention.

A review of the application shows that the Applicants had possession of the polypeptides that they are claiming *before* having possession of the nucleic acid on which the Patent Office has focused. Examples 4-5 of the application teach that an Flt4 ligand was isolated and purified from the conditioned media from the PC-3 prostatic adenocarcinoma cell line. (Specification, pages 15-19.) An affinity purification procedure was described that employed the Flt4 extracellular domain, also described in the application. (See Examples 3-5.) The purified polypeptide had a molecular weight of approximately 23 KD (SDS-PAGE, reducing conditions) and stimulated Flt4 phosphorylation. (See specification, paragraph bridging pages 18-19.) The polypeptide was sufficiently pure to determine an amino-terminal sequence which is set forth in SEQ ID NO: 13. (See specification at page 19, first full paragraph.) The purpose of the written description requirement is to ensure that inventors are in possession of what they are claiming, and when one considers the teachings of the Applicants examples, summarized above, it is abundantly clear to the reader that the Applicant was in possession of what is being claimed herein (e.g., in claims 45 and 51).<sup>3</sup>

In the rejection, the Patent Office also examines whether the claims explicitly recite "limitations to provide function":

---

<sup>3</sup> Of course, the Application goes on to describe many more features of the invention, including the isolation of a cDNA encoding the protein, deduced amino acid sequence, and other properties. The entirety of the application supports broad genus claims which the Applicants intend to pursue in this application or related applications.

B. The rejection of claims 13, 16, 17, 20-22, 25, and 28 should be withdrawn.

Claims 45 and 51 (which correspond with rejected claims 16 and 17) are the two independent claims of the remaining claims that stand rejected (13, 16, 17, 20-22, 25, and 28). As set forth below, the rejection of these claims (and their dependent claims) also should be withdrawn.

As part of the basis for its rejection, the Patent Office expressed concern that the claims encompassed non-human polypeptides and anti-flt4 antibody peptides. While the Applicants believe they are entitled to claim both of these categories of polypeptides in their patent applications, these concerns are moot with respect to the rejected claims. Claim 45 (which replaces claim 16) recites "A purified and isolated polypeptide comprising a human polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13." The recitation "human" negates the Patent Office's allegation that the claim encompasses polypeptides from other species, and the recitation of the amino sequence in SEQ ID NO: 13 would reasonably be expected to exclude anti-Flt4 antibodies, because the sequence of SEQ ID NO: 13 was not obtained from an antibody.

Claim 51, which replaces claim 17, recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, wherein said polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." The Applicants respectfully submit that non-human polypeptides and anti-Flt4 antibodies are not purifiable from the PC-3 cell line.

The Patent Office observed that, "In making a determination of whether the application complies with the written description requirement of 35



New claims 30, 32, 33, and 47 are dependent claims analogous to other dependent claims that had been pending in the application. (See Table.) Claims 31, 34, 37, 39, 44, 46, 49, 53, 56, and 57 are dependent method claims which the Applicants request to have favorably considered with their parent polypeptide and composition claims, upon allowance of those claims, consistent with the Patent Office's policy announced at 1184 OG 86 (March 26, 1996). Support for the dependent process claims is found throughout the application, including at pages 6-7.

III. **The Patent Office's rejection of claims 1, 13-17, 19, 20-22, 23, 25, and 28 under 35 U.S.C. §112, first paragraph, for lack of written description should be withdrawn.**

The Patent Office rejected claims 1, 13-17, 19, 20-22, 23, 25, and 28 under 35 U.S.C. §112, first paragraph, alleging that these claims contain subject matter which was not described in the specification in a way that reasonably conveys to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The Applicants respectfully traverse.

A. The rejection of claims 1, 14, 15, 19, and 23 has been rendered moot.

The scope of the rejection has been narrowed by the amendments set forth above. Specifically, the Applicants have canceled rejected claims 1 and 23.<sup>2</sup> Several of the other rejected claims (e.g., claims 14, 15, 19 -- now embodied in claims 33, 38, 40, 41, and 47-- have been amended to depend (directly or indirectly) from claims 29 and 35 (which are similar to claims 8 and 9), which the Patent Office had acknowledged contain sufficient limitations to satisfy the written description requirement). The rejection of claims 14, 15, and 19 should therefore be withdrawn.

---

<sup>2</sup> The Patent Office's main allegation in support of its rejection is that "The broadest claim only requires the polypeptide to be capable of binding to the extracellular domain of human Flt4 receptor tyrosine kinase. The additional limitations of the dependent claims fail to provide the structure which is required for this receptor binding or for receptor activation (an additional functional limitation)." (Office action at p. 3.) As explained above, claim 1 (the claim that required only binding) has been canceled without prejudice.

Current Claim	Previous Claim	Comments
45	16	Amended to recite "human"
45	New	Method of using elected product
47	14	
47	25	
49	New	Method of using elected product
50	28	
51	17 & 13	
52	20	
53	New	Method of using elected product
54	21	
55	22	
56	New	Method of using elected product
57	New	Method of using elected product

## II. Explanation of amendments

Most of the amendments to the claims merely represent a consolidation of the claims for the purpose of conciseness and clarity. For example, words or phrases that have been added to claims have, for the most part, been taken from other claims that have been canceled. Many of the dependent claims are repetitive (but depend from different independent claims).

The amendment to independent claim 45 (formerly 16) to recite "human" finds support throughout the application, because Examples in the application describe the isolation and characterization of a human cDNA and protein.

The Applicants do not intend by these or any other amendments to abandon the subject matter of any claim as originally filed or later presented, and reserve the right to pursue such subject matter in continuing applications.

soon become available." After numerous written and telephonic status inquiries by the undersigned attorney, the Patent Office resumed prosecution and issued the outstanding Office Action.

In the present amendment, the Applicants cancel all pending claims;<sup>1</sup> and add new claims 29-57. Thus, upon entry of the foregoing amendments, claims 29-57 are pending. The following table correlates new and old claim numbers.

Current Claim	Previous Claim	Comments
29	8	Amended to clarify that claimed polypeptide does not include a large carboxy-terminal portion of SEQ ID NO: 33
30	12	
31	New	Method of using elected product
32	19	
33	14	
34	New	Method of using elected product
35	9	Amended to include limitations relating to high affinity binding and amino acid sequence (SEQ ID NO: 33)
36	12	
37	New	Method of using elected product
38	14	
39	New	Method of using elected product
40	15	
41	19	
42	26	
43	27	
44	New	Method of using elected product

<sup>1</sup> In an interview with the undersigned on June 22, 2000, the Examiner requested that the claims be presented in a renumbered claim set.

<sup>24</sup>  
52. A polypeptide according to claim <sup>23</sup>51 which is capable of stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

<sup>25</sup>  
53. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim <sup>24</sup>52.

<sup>26</sup>  
54. A polypeptide according to claim <sup>23</sup>51 further comprising a detectable label.

<sup>27</sup>  
55. A pharmaceutical composition comprising a polypeptide according to claim <sup>23</sup>51 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

<sup>28</sup>  
56. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim <sup>27</sup>55.

<sup>29</sup>  
57. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim <sup>23</sup>51.

#### REMARKS

##### **I. Prosecution History.**

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In an amendment dated February 10, 1997, the applicants canceled claims 10 and 18, amended claims 1, 8, 9, 14, and 17, and added new claims 20-25. In an amendment dated June 11, 1997, the applicants canceled claims 3-7 and 11, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28. Thereafter, the Patent Office suspended prosecution for more than 2½ years on the grounds that a relevant reference "may

<sup>17</sup>  
~~46~~. A purified and isolated polypeptide comprising a human polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said human polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

<sup>18</sup>  
~~46~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ~~46~~.

<sup>19</sup>  
~~47~~. A purified and isolated polypeptide according to claim <sup>17</sup>~~46~~ that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

<sup>20</sup>  
~~48~~. A pharmaceutical composition comprising a polypeptide according to claim <sup>17</sup>~~46~~ in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

<sup>21</sup>  
~~48~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim <sup>20</sup>~~48~~.

<sup>22</sup>  
~~50~~. A polypeptide according to claim <sup>17</sup>~~46~~ further comprising a detectable label.

<sup>23</sup>  
~~51~~. A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, wherein said polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions and is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

<sup>8</sup>  
~~36~~. A pharmaceutical composition comprising a polypeptide according to claim ~~36~~<sup>7</sup> in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

<sup>9</sup>  
~~37~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim ~~36~~<sup>8</sup>.

<sup>10</sup>  
~~38~~. A purified and isolated polypeptide according to claim ~~36~~<sup>7</sup> that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.

<sup>11</sup>  
~~39~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ~~38~~<sup>10</sup>.

<sup>12</sup>  
~~40~~. A purified and isolated polypeptide according to claim ~~38~~<sup>10</sup>, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

<sup>13</sup>  
~~41~~. A polypeptide according to claim ~~36~~<sup>7</sup> further comprising a detectable label.

<sup>14</sup>  
~~42~~. A polypeptide according to claim ~~36~~<sup>7</sup> wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

<sup>15</sup>  
~~43~~. A polypeptide according to claim ~~36~~<sup>7</sup> wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

<sup>16</sup>  
~~44~~. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim ~~36~~<sup>7</sup>.

AMENDMENTS

In the claims:

Please cancel all pending claims 1-2, 8-9, 12-17, and 19-28; and add new claims 29-57 as shown below:

- 1  
29. A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of amino acids 1-180 of SEQ ID NO: 33 effective to permit such binding, said polypeptide lacking all of amino acids of SEQ ID NO: 33 beyond position 180.
- 2  
30. A pharmaceutical composition comprising a polypeptide according to claim 29 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.
- 3  
31. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising administering to a person in need of modulation of Flt4 receptor tyrosine kinase activity a composition according to claim 30.
- 4  
32. A polypeptide according to claim 29 further comprising a detectable label.
- 5  
33. A purified and isolated polypeptide according to claim 29 that binds Flt4 and stimulates Flt4 phosphorylation in mammalian cells expressing Flt4.
- 6  
34. A method of modulating the activity of human Flt4 receptor tyrosine kinase comprising contacting cells that express human Flt4 receptor tyrosine kinase with a polypeptide according to claim 29.
- 7  
35. A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase (Flt4), wherein the polypeptide comprises a portion of SEQ ID NO: 33 effective to permit such binding, and wherein the polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.



#29D 8/10/00  
T6/04  
PATENT  
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.	)	I hereby certify that this paper is being
	)	deposited with the United States Postal
Serial No: 08/510,133	)	Service with sufficient postage as first
	)	class mail, postage prepaid, in an
Filed: August 1, 1995	)	envelope addressed to: Assistant
	)	Commissioner for Patents, Washington,
Title: Receptor Ligand	)	D.C., 20231 on this date:
	)	
Group Art Unit: 1646	)	Date: July 24, 2000
	)	
Examiner: Christine Saoud	)	<u>David A. Gass</u>
	)	David A. Gass
	)	Registration No. 38,153
	)	Attorney for Applicants

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

In an Office action mailed April 26, 2000, the Patent Office rejected claims 1, 2, 8, 9, 12-17, and 19-28 variously under 35 USC §102 and 112, first paragraph. The Applicants respectfully request reconsideration in light of the following amendments and remarks.

37726/2000 W08H11 00000035 08510133  
01 FC:203 54.00 BP



1. **Small Entity Status**

Verified statement(s) claiming small entity status is(are) attached.

- ☒ Small entity status has been established and is still effective.  
 Has not been established.

2. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	29	MINUS	23	= 6	X 9=	\$54.00	X18=	\$
INDEP.	4	MINUS	5	= 0	X39=	\$0	X78=	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim					+130=	\$	+260=	\$
TOTAL ADDITIONAL FEE						\$54.00	OR	\$

3. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$54.00  
☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
 A copy of this Transmittal is enclosed.

4. **Deposit Account and Refund Authorization**

- ☒ The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.  
☒ Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
 MURRAY & BORUN  
 6300 Sears Tower  
 233 South Wacker Drive  
 Chicago, Illinois 60606-6402  
 (312) 474-6300

By: 

David A. Gass  
 Reg. No: 38,153

July 24, 2000



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	)	Title: RECEPTOR LIGAND
	)	
Alitalo et al.	)	
	)	
Serial No: 08/510,133	)	Group Art Unit: 1646
	)	
Filed: August 1, 1995	)	Examiner: Christine Saoud

AMENDMENT TRANSMITTAL

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith is an amendment for the above application.

---

**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 24, 2000, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
\_\_\_\_\_  
David A. Gass

#27  
1/27

PATENT  
28967/32863

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): Alitalo et al. )  
Serial No: 08/510,133 )  
Filed: August 1, 1995 )  
Title: Receptor Ligand )  
Group Art Unit: 1646 )  
Examiner: Christine Saoud )

ASSOCIATE POWER OF ATTORNEY

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

The undersigned attorney of record in the above-identified application  
hereby appoints as associate attorney(s):

Frank S. DiGiglio (Reg. No. 31,346)  
Scully, Scott, Murphy & Presser  
400 Garden City Plaza  
Garden City, New York 11530  
(516) 742-4343

to prosecute this application, to make alterations or amendments therein, and to  
transact any and all business in the Patent and Trademark Office connected  
therewith.

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN



David A. Gass  
Registration No. 38,153

June 22, 2000

<b>Office Action Summary</b>	Application No. <b>08/510,133</b>	Applicant(s) <b>ALITALO et al.</b>
	Examiner <b>Christine Saoud</b>	Group Art Unit <b>1646</b>

☒ Responsive to communication(s) filed on Jun 11, 1997

☐ This action is FINAL.

☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

**Disposition of Claims**

☒ Claim(s) 1, 2, 8, 9, 12-17, and 19-28 is/are pending in the application.

Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.

☐ Claim(s) \_\_\_\_\_ is/are allowed.

☒ Claim(s) 1, 2, 8, 9, 12-17, and 19-28 is/are rejected.

☐ Claim(s) \_\_\_\_\_ is/are objected to.

☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.

**Application Papers**

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.

☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

**Attachment(s)**

☐ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 25

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILED DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
081510.133			

EXAMINER
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ART UNIT	PAPER NUMBER
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27

DATE MAILED:

#### INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) Christine Saoud (3) DAVID GASS  
(2) Gary Kunz (4) WILLIAM K. MERKEL  
Date of Interview June 22, 2000 (5) Frank S. DiCoroglio

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☒ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description: \_\_\_\_\_

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: 8, 9, 16, 17

Identification of prior art discussed: of record in case (Hu et al.)

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: Discussed above clms in terms of prior art of record. Clm 9 & 16 & 17 distinguishes over the prior art of record. Additional limit to clm 8 of M.W. or C-terminal truncation would also distinguish over the prior art.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

FORM PTOL-413 (REV.1-96)

Christine Saoud



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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08/510,133	08/01/95	SCULLY	28115/32863
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FRANK S. DIGIGLIO  
SCULLY SCOTT MURPHY & PRESSER  
400 GARDEN CITY PLAZA  
GARDEN CITY NY 11530

HY22-0829

EXAMINER

SAVED C

ART UNIT	PAPER NUMBER
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1647

28

DATE MAILED:

06/29/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/510,133	08/01/95	ALITALO	28113/32863

HM12/0426  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

EXAMINER
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SAGUD, C

ART UNIT	PAPER NUMBER
1645	26

DATE MAILED: 04/26/00

Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks



Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
cl	C134	Genbank H96533, "yw04b12.r1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996
	C135	Genbank T81690, "yd29f07.r1 Homo sapiens cDNA clone 109669 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995
	C136	Genbank T84377, "yd37h08.r1 Homo sapiens cDNA clone 110463 5' similar to SP:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3," Deposited by Hillier, L. <i>et al.</i> Dated 16-Mar-1995
	C137	Genbank N42368, "yy15b11.r1 Homo sapiens cDNA clone 271293 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C138	Genbank N42374, "yy15d11.r1 Homo sapiens cDNA clone 271317 5'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Jan-1996
	C139	Genbank H81868, "yv83d09.s1 Homo sapiens cDNA clone 249329 3'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C140	Genbank H81867, "yv83d09.r1 Homo sapiens cDNA clone 249329 5'," Deposited by Hillier, L. <i>et al.</i> Dated 09-Nov-1995
	C141	Genbank AA149461, "z127h03.r1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 5' similar to SW:BAR3_CHITE Q03376 BALBIANI RING PROTEIN 3 PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 14-May-1997
	C142	Genbank R77495, "yi79e04.s1 Homo sapiens cDNA clone 145470 3'," Deposited by Hillier, L. <i>et al.</i> Dated 07-Jun-1995
	C143	Genbank H07899, "y186g06.s1 Homo sapiens cDNA clone 45138 3'," Deposited by Hillier, L. <i>et al.</i> Dated 23-Jun-1995
	C144	Genbank T89295, "yd37h08.s1 Homo sapiens cDNA clone 110463 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Mar-1995
cl	C145	Genbank C21512, "HUMGS0010510, Human Gene Signature, 3'-directed cDNA sequence," Deposited by Okubo, K. Dated 01-Oct-1996

EXAMINER C. Saoud	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510.133
<b>INFORMATION DISCLOSURE STATEMENT</b> <i>(Use several sheets if necessary)</i>		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)			
CA	C122	Genbank N94399, "zb76f04.s1 Soares senescent fibroblasts NbHSF Homo sapiens cDNA clone 309535 3'," Deposited by Hillier, L. <i>et al.</i> Dated 20-Aug-1996	
	C124	Genbank H05177, "y185b08.r1 Homo sapiens cDNA clone 44993 5'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995	
	C124	Genbank AA479987, "zv18h12.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 3'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997	
	C125	Genbank H05134, " y185b08.s1 Homo sapiens cDNA clone 44993 3'," Deposited by Hillier, L. <i>et al.</i> Dated 21-Jun-1995	
	C126	Genbank, AA298182 "EST113866 Bone VII Homo sapiens cDNA 5' end," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997	
	C127	Genbank AA298283, "EST113896 Bone VII Homo sapiens cDNA 5' end similar to similar to vascular endothelial growth factor," Deposited by Adams, M.D. <i>et al.</i> Dated 18-Apr-1997	
	C128	Genbank T81481, "yd29f07.s1 Homo sapiens cDNA clone 109669 3'," Deposited by Hillier, L. <i>et al.</i> Dated 15-Mar-1995	
	C129	Genbank AA425303, "zw46b06.s1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 3', mRNA sequence," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997	
	C130	Genbank Z40230, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995	
	C131	Genbank Z44272, "H. sapiens partial cDNA sequence; clone c-1wf11," Deposited by Genexpress. Dated 21-Sep-1995	
	C132	Genbank AA478766, " zv18h12.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 754055 5'," Deposited by Hillier, L. <i>et al.</i> Dated 08-Aug-1997	
CA	C133	Genbank H96876, "yw04b12.s1 Soares melanocyte 2NbHM Homo sapiens cDNA clone 251231 3'," Deposited by Hillier, L. <i>et al.</i> Dated 25-Nov-1996	

EXAMINER C. Seand	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Any. Docket No. 28967/32863	Serial No. 08/510,133
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, K. <i>et al.</i>	
		Filing Date August 1, 1995	Group 1646

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)			
ca	C112	Achen, M.G. <i>et al.</i> , "Vascular Endothelial Growth Factor D (VEGF-D) is a Ligand for the Tyrosine Kinases VEGF Receptor 2 (Flk1) and VEGF Receptor 3 (Flt4)," <i>Proceedings of the National Academy of Science, USA</i> , 95:548-553 (January, 1998).	
	C113	Adams, M.D. <i>et al.</i> , "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence," <i>Nature</i> , 377(6547 Supplement):3-174 (September, 1995).	
	C114	Cohen, T. <i>et al.</i> , "VEGF121, A Vascular Endothelial Growth Factor (VEGF) Isoform Lacking Heparin Binding Ability, Requires Cell-Surface Heparan Sulfates for Efficient Binding to the VEGF Receptors of Human Melanoma Cells," <i>Journal of Biological Chemistry</i> , 270(19):11322-11326 (May 12, 1995).	
	C115	Genbank AA151613, "z127h03.s1 Soares pregnant uterus NbHPU Homo sapiens cDNA clone 503189 3'," Hillier, L. <i>et al.</i> , Dated 14-May-1997	
	C116	Genbank AA425486, "zw46b06.r1 Soares total fetus Nb2HF8 9w Homo sapiens cDNA clone 773075 5' similar to SW:VEGF MOUSE Q00731 VASCULAR ENDOTHELIAL GROWTH FACTOR PRECURSOR," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997	
	C117	Genbank N31713, "yy15b12.s1 Homo sapiens cDNA clone 271295 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996	
	C118	Genbank N31720, "yy15d12.s1 Homo sapiens cDNA clone 271319 3'," Deposited by Hillier, L. <i>et al.</i> Dated 10-Jan-1996	
	C119	Genbank AA406492, "zv12g06.r1 Soares NhHMPu S1 Homo sapiens cDNA clone 75366 5'," Deposited by Hillier, L. <i>et al.</i> Dated 17-May-1997	
	C120	Genbank N50972, "yy94b08.s1 Homo sapiens cDNA clone 281175 3'," Deposited by Hillier, L. <i>et al.</i> Dated 14-Feb-1996	
ca	C121	Genbank AA421713, "zu24b03.s1 Soares NhHMPu S1 Homo sapiens cDNA clone 738893 3'," Deposited by Hillier, L. <i>et al.</i> Dated 16-Oct-1997	

EXAMINER C. Saoud	DATE CONSIDERED 4/12/00
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

# 15/11/95  
SHEET 1 of 5

**FILE COPY**

Form PTO-1449 (Modified) U.S. Department of Commerce  
Patent and Trademark Office

OCT 28 1993

**INFORMATION DISCLOSURE STATEMENT**

(Use several sheets if necessary)

Any. Docket No. 28967/32863	Serial No. 08/510,133
Applicant Alitalo, K. <i>et al.</i>	
Filing Date August 1, 1995	Group 1646

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate
CA	A1	08/207,550	none	Jing-Shan Hu <i>et al.</i>			03/08/94
CA	A2	08/465,968	none	Crain Rosen <i>et al.</i>			06/06/95
CA	A3	60/003,491	none	James Lee <i>et al.</i>			09/08/95
CA	A4	08/554,374	none	Lyman, S.			11/08/95
CA	A5	5,326,695	07/05/94	Andersson <i>et al.</i>	435	70.1	
CA	A6	5,932,540	08/03/99	Jing-Shan Hu <i>et al.</i>	514	2	
CA	A7	5,935,820	08/10/99	Jing-Shan Hu <i>et al.</i>	435	69.4	

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
CA	B7	0 506 477 A1	03/27/92	EP				
CA	B8	97/05250 A	02/13/97	WO				
CA	B9	97/09427 A	03/13/97	WO				
CA	B10	97/17442 A	05/15/97	WO				

EXAMINER <i>C. Saoud</i>	DATE CONSIDERED <i>4/12/00</i>
<p>*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.</p>	

Documents B8-B10, C112, C114, and C149-C152 were identified by the European Patent Office in an International Search Report for a related PCT application. A copy of the search report is also attached hereto.

Documents C115-C148 pertain to sequences, such as EST's, that have been posted in the Genbank Database, where the sequences should be available in computer readable form.

This Supplemental Information Disclosure Statement is not intended to be an admission that a search has been made, that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

The Commissioner is authorized to charge any fee required by this paper to Deposit Account No. 13-2855. A copy of this paper is enclosed.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

October 26, 1999

By:



David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



Attorney Docket No. 28967/32863

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Kari Alitalo ) I hereby certify that this paper and the  
and Vladimir Joukov ) documents referred to as enclosed  
Serial No.: 08/510,133 ) herewith are being deposited with the  
Filed: August 1, 1995 ) United States Postal Service as First  
For: RECEPTOR LIGAND ) Class Mail, postage prepaid, in an  
Group Art Unit: 1646 ) envelope addressed to: Assistant  
Examiner: Saoud, C. ) Commissioner for Patents,  
Washington, DC 20231, on this date:  
October 26, 1999  
David A. Gass  
Reg. No.: 38,153  
Attorney for Applicants

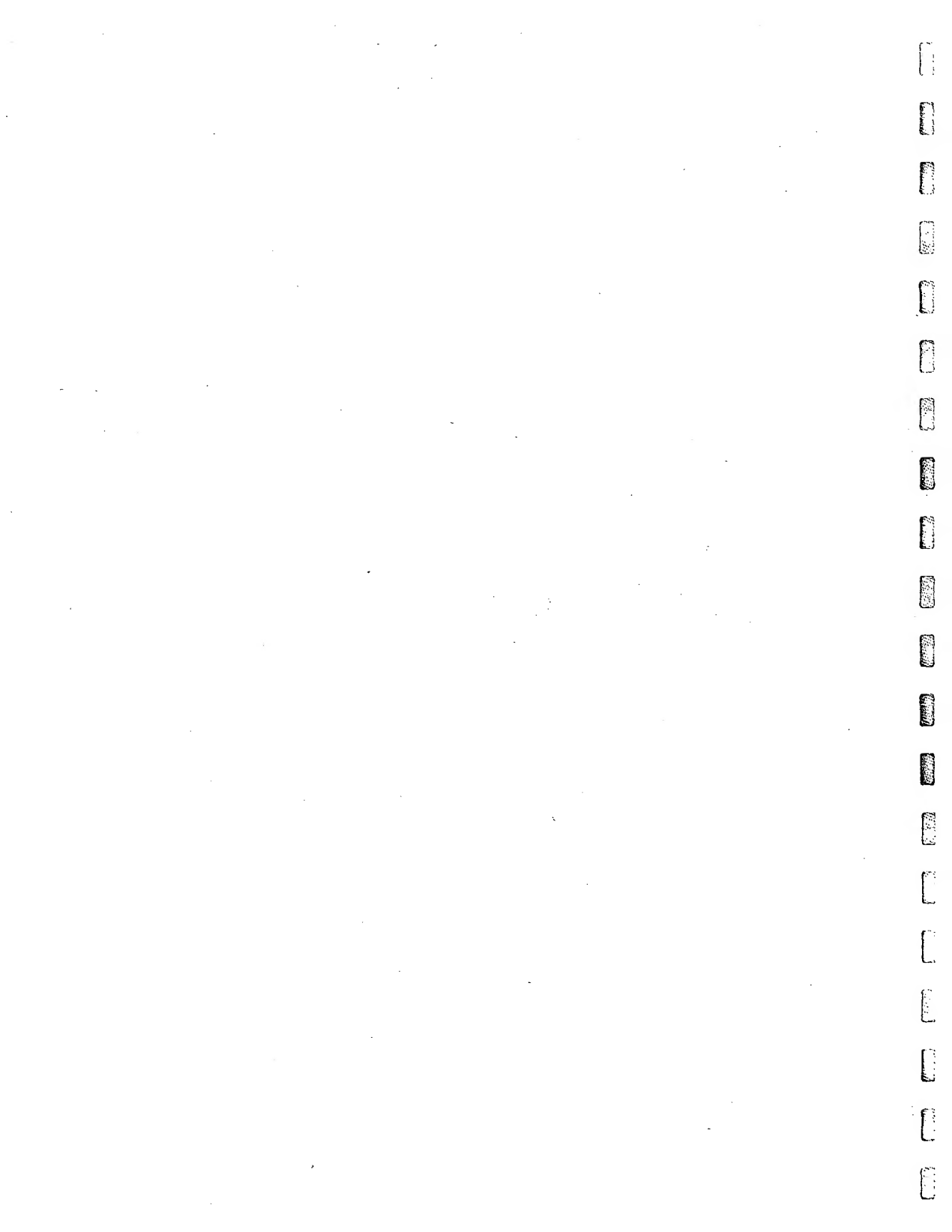
SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Submitted herewith are a Form PTO-1449 listing several documents, together with a copy of each listed document. The Applicants respectfully request that these documents be made of record and considered by the Examiner in the above-identified application.

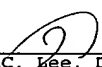
Documents A1-A4 are U.S. priority documents of published PCT applications that are now publically available from WIPO.



In re Application of  
KARI ALITALO, ET AL.  
Serial No. 08/510,133  
Filed: August 1, 1995  
For: RECEPTOR LIGAND

:  
:  
: SUSPENSION  
: OF PROSECUTION  
:

A reference relevant to the examination of this application will soon become available. Ex parte prosecution is SUSPENDED pending the availability of the reference. Applicants will be notified when the reference becomes available and prosecution will resume at that time.

  
Mary C. Lee, Deputy Director  
Patent Examining Group 1800

MARSHALL O'TOOLE GERSTEIN MURRAY  
AND BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO, IL 60606-6402



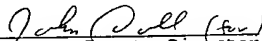


UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

In re Application of  
KARI ALITALO, ET AL.  
Serial No. 08/510,133  
Filed: August 1, 1995  
For: RECEPTOR LIGAND

:  
:  
: SUSPENSION  
: OF PROSECUTION  
:

A reference relevant to the examination of this application will soon become available. Ex parte prosecution is SUSPENDED pending the availability of the reference. Applicants will be notified when the reference becomes available and prosecution will resume at that time.

  
Mary C. Lee, Deputy Director  
Patent Examining Group 1800

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AND BORUN  
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CHICAGO, IL 60606-6402



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/510,133	08/01/97	AL17610	08113/32863

HM11/0403  
MARSHALL O'TOOLE GERSTEIN MURRAY  
AND BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-8402

EXAMINER	
SEARCHED	
ART UNIT	PAPER NUMBER
1646	23

DATE MAILED:

04/03/98

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents

IN RESPONSE TO APPLICANT'S LETTER OF STATUS INQUIRY,  
FILED 12/24/97, SEE ATTACHED.



**PATENT**  
**28967/32863**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Application of:	)	Title: RECEPTOR LIGAND
	)	
Alitalo et al.	)	
	)	
Serial No. 08/510,133	)	Art Unit: 1801
	)	
Filed: August 1, 1995	)	Examiner: Lathrop, B.
	)	

Change of Inventor's Address

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Please be advised that the residence and mailing address of co-inventor Vladimir Joukov is now as follows:

51 Massachusetts Avenue, Apt. 1F  
Boston, Massachusetts 02115

This notification is NOT intended as a change of correspondence address. Please continue to send correspondence to the Applicants' attorney at the address below:

Respectfully submitted,  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: *David A. Gass*  
David A. Gass  
Registration No. 38,153

Date: Feb 24, 1998

## ASSIGNMENT

WHEREAS Helsinki University Licensing, Ltd., Viikinkaari 8 A, FIN-00710 Helsinki, Finland (hereinafter HUL), its successors and assigns, is the assignee of the entire right, title and interest in the invention or improvements of Kari Alitalo and Vladimir Joukov relating to the cloning, isolation and sequencing of human Vascular Endothelial Growth Factor C (VEGF-C) disclosed in certain applications for Letters Patent of the United States, and in said applications and any and all other applications, both United States and foreign, which Kari Alitalo and Vladimir Joukov may file, either solely or jointly with others, on said invention or improvements, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said applications, and in any reissue or extension thereof; and

WHEREAS, for ten dollars (\$10.00), and other good and valuable consideration enumerated in a written agreement dated 24 October 1996, the sufficiency of which is hereby acknowledged, HUL has agreed to share ownership of the aforementioned inventions improvements, applications, patents, reissues, extensions, and the like on a 50% / 50% equal basis with Ludwig Institute for Cancer Research, a Swiss not-for-profit corporation having an office at 1345 Avenue of the Americas, New York, New York 10105, United States of America (hereinafter LICR);

NOW, THEREFORE, HUL hereby assigns to LICR a fifty percent (50%) interest in the patent applications identified in the following LIST OF PATENT PROPERTIES, and in any and all Letters Patent of the United States and foreign countries, which may be obtained on any of said patent applications, and in any reissue or extension thereof.

### LIST OF PATENT PROPERTIES

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>
08/510,133	01/08/95	Receptor Ligand
08/585,895	12/01/96	Receptor Ligand
08/601,132	14/02/96	Receptor Ligand
08/671,573	28/06/96	Receptor Ligand VEGF-C
PCT/FI96/00427	01/08/96	Receptor Ligand VEGF-C
08/795,430	02/05/97	Vascular Endothelial Growth Factor C (VEGF-C) Protein and Gene, Mutants Thereof, and Uses Thereof

WITNESS my hand this 25 day of April, Nineteen Hundred and Ninety-Seven.

Witnesses:

1) [Signature]  
Name:

2) [Signature]  
Name:

Helsinki University  
Licensing, Ltd.

By: [Signature]  
Heikki Lampi  
President



## POWER OF ATTORNEY

Helsinki University Licensing, Ltd., hereby appoints:

Alvin D. Shulman (19,412)	Timothy J. Venezia (26,348)	Richard A. Schnurr (30,890)	James J. Napoli (32,361)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Anthony Nimmo (30,920)	Richard M. La Barge (32,254)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Christine A. Dudzik (31,245)	Karl A. Vick (33,288)
Nate F. Scarpelli (22,320)	Patrick D. Ertel (26,877)	Kevin D. Hogg (31,839)	Douglas C. Hochstetler (33,710)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	Robert M. Gerstein (34,824)
Trevor B. Joike (25,542)	David A. Gass (38,153)		

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel &amp; Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 Receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
United States of America  
(312) 474-6300

Helsinki University Licensing, Ltd.  
Viikinkaari 8 A  
FIN-00710 Helsinki  
FINLAND

(Date)

28th of June 1998

By:

Name: Heikki Lampi

Title: President



## POWER OF ATTORNEY

The Ludwig Institute for Cancer Research hereby appoints:

Alvin D. Stulman (19,412)	Timothy J. Vezau (26,348)	Richard A. Schnurr (30,890)	James J. Napoli (32,361)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Anthony Nimmo (30,920)	Richard M. La Berge (32,254)
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Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	Robert M. Gerstein (34,824)
Trevor B. Joike (25,542)	David A. Gass (38,153)		

as its attorneys, with full powers of substitution and revocation, to act on its behalf before the U.S. Patent and Trademark Office in connection with the following applications filed by Kari Alitalo et al. of which it is an assignee:

<u>Application No.</u>	<u>Filing Date</u>	<u>Title</u>	<u>Assignment Reel &amp; Frame #</u>
08/510,133	01/Aug/95	Receptor Ligand	8378/0566
08/585,895	12/Jan/96	Receptor Ligand	8145/0829
08/601,132	14/Feb/96	Antibodies Reactive with VEGF-C, a Ligand for the Flt4 receptor Tyrosine Kinase (VEGFR-3)	8129/0688
08/671,573	28/Jun/96	Receptor Ligand VEGF-C	8161/0909

Please continue to send correspondence to:

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
United States of America  
(312) 474-6300

Ludwig Institute for Cancer Research  
1345 Avenue of the Americas  
New York, New York 10105

(Date) 26-01-98

By: 

Name: A. Murray

Title: ASSOCIATE DIRECTOR

Please enter the power of attorney documents into the file for the above-identified patent application.

Respectfully submitted,  
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: *David A. Gass*  
David A. Gass  
Registration No. 38,153

Date: Feb 24, 1998



G/K-1801  
1652  
#2  
MC  
PATENT  
28967/32863  
04/10/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	I hereby certify that this paper is
Alitalo et al.	)	being deposited with the United
Serial No. 08/510,133	)	States Postal Service as first class
Filed: August 1, 1995	)	mail, postage prepaid, in an
For: RECEPTOR LIGAND	)	envelope addressed to: Assistant
Art Unit: 1801	)	Commissioner for Patents,
Examiner: Lathrop, B.	)	Washington, D.C. 20231, on this
	)	date:
	)	Dated: Feb 24, 1995
	)	<u>David A. Gass</u>
	)	David A. Gass

16X-125  
NO 1/95

TRANSMITTAL OF POWERS OF ATTORNEY

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Transmitted herewith are power of attorney documents executed by the two assignees of the above-identified patent application: Helsinki University Licensing, Ltd., and The Ludwig Institute for Cancer Research.

The above-identified application was assigned by the inventors to Helsinki University Licensing, Ltd., (HUL) in an assignment recorded at Reel 8378, Frame 0566.

HUL assigned a 50% interest in the application to The Ludwig Institute for Cancer Research, as evidenced by the attached assignment document which has been submitted for recordation.



Dec. 29, 1997 3:37PM MARSHALL, O'TOOLE

No. 4468 P. 1/2

From: 0808

**MARSHALL, O'TOOLE, GERSTEIN, MURRAY & BORUN**

ATTORNEYS AT LAW  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO, ILLINOIS 60606-6402  
(312) 474-6300  
FAX: (312) 474-0448

#22  
28  
01/07/96

December 29, 1997

**FACSIMILE TRANSMITTAL SHEET**

TO: Assistant Commissioner for Patents  
1-703-305-7401

CLIENT NO: 28967  
MATTER NO: 32863  
COUNTRY CODE: US

FROM: David A. Gass

RECEIVED  
DEC 29 1997  
GROUP 1000

PAGES (INCLUDING THIS PAGE): 2

PLEASE CONFIRM RECEIPT: Yes

MESSAGE:

*Please contact Muriel Gallaher at 312-474-6808 if you do not receive all of the pages in good condition.*

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Dec. 29. 1997 3:37PM MARSHALL, O'TOOLE

No. 4468 P. 2/2  
From: 0808

PATENT APPLICATION  
DOCKET NO. 28967/32863

IN THE UNITED STATES PATENT  
AND TRADEMARK OFFICE

Application of:	)	<b>Certificate of Facsimile Transmittal</b>
	)	
Alitalo et al.	)	I hereby certify that this paper is being
	)	transmitted via facsimile to the Assistant
Serial No: 08/510,133	)	Commissioner for Patents, Washington, D.C.
	)	20231, on this date:
Filed: August 1, 1995	)	
	)	Date: <u>Monday, December 29, 1997</u>
For: RECEPTOR LIGAND	)	
	)	Facsimile No. 1-703-305-7401
Group Art Unit: 1801	)	
	)	<u>David A. Gass</u>
Examiner: Lathrop, B	)	David A. Gass

STATUS INQUIRY

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In a communication dated June 25, 1997, the Patent Office suspended prosecution for six months because "a reference relevant to the examination of this application may soon become available." The Applicants were advised to make a status inquiry upon expiration of the six month period. Please advise as to the status of this application at your earliest convenience.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:

David A. Gass  
David A. Gass  
Reg. No: 38,153


Date:

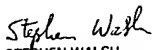
12/29/97

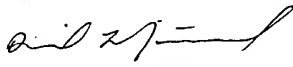
has been entered.

*Suspension of Prosecution*

6. A reference relevant to the examination of this application may soon become available. *Ex parte* prosecution is **SUSPENDED FOR A PERIOD OF 6 MONTHS** from the date of this letter. Upon expiration of the period of suspension, applicant should make an inquiry as to the status of the application.

  
Brian Lathrop, PhD  
6/24/97

  
STEPHEN WALSH  
SUPERVISORY PATENT EXAMINER  
GROUP 1800

  
DAVID L. FITZGERALD  
PRIMARY EXAMINER  
GROUP 1800



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

DEAFCE-1994

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
Brian Lathrop	
ART UNIT	PAPER NUMBER
1801	21
DATE MAILED:	

Please find below a communication from the EXAMINER in charge of this application  
Commissioner of Patents

*Priority*

1. Applicant correctly contends that the adverse determination of priority under 35 USC 120 is inappropriate in the absence of intervening prior art used in a rejection (part D of Paper No. 20), and it is therefore **moot and withdrawn as premature**. Applicant has met the formal requirements for priority under 35 USC 120 to US Serial No. 08/340011.
2. Receipt of the declaration under 37 CFR 1.132 filed 6/16/97, Paper No. 20, is acknowledged. The declaration has entered but not considered because the adverse determination of priority under 35 USC 120 to which it is addressed is moot.

*Double Patenting*

3. Co-pending application Serial No. 08/671573, which has recently come to the examiner's attention, claims subject matter which substantially overlaps the instantly claimed subject matter. *New rejections will be set forth in the next office action. BT*

*Withdrawal of Finality*

4. Because new rejections based on the judicially created doctrine of provisional double patenting will be required, the finality of the previous Office action is **withdrawn**.

*Entry of Amendment*

5. Receipt of the amendment filed 6/16/97, Paper No. 20, is acknowledged. The amendment

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label.

16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

19. A polypeptide according to claim 1 further comprising a detectable label.

20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. (Amended) A polypeptide according to claim 17 further comprising a detectable label.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

**Exhibit B**

1. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an amino acid sequence shown in SEQ ID NO: 33.

8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

9. (Twice amended) A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

12. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

13. A polypeptide according to claim 1 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

122. Betsholtz, C., and Heldin, C.-H. (1997). Platelet-derived growth factor: A regulator of connective tissue development and reaction. In: *Wennergren Symposia Series*, Portland Press, London, (in press).
123. Miyazono, K., ten Dijke, P., Souchelnytskyi, S., Nakao, A., Imamura, T., Hanai, J.-i., Kawabata, M., and Heldin, C.-H. (1997). Transforming growth factor- $\beta$  receptors and signal transduction. In: *Serono Symposia on "Inhibin, activin and follistatin; Recent advances and future views"* (Aono, T., Sugino, H., and Vale, W.W., eds.), (in press).
124. Heldin, C.-H. (1997). Simultaneous induction of stimulatory and inhibitory signals by PDGF. *FEBS Lett.* (in press).
125. Heldin, C.-H. (1997). Members of the transforming growth factor- $\beta$  superfamily signal through serine/threonine kinase receptors. *Japanese J. Cancer Res.* (in press).



104. Heldin, C.-H., and Claesson-Welsh, L. (1994). Receptors for platelet-derived growth factor (PDGF). In: *Guidebook to Cytokines and their Receptors* (Nicola, N., ed.), Oxford University Press, Oxford, pp. 205-207.
105. Westermark, B., Heldin, C.-H., and Nistér, M. (1995). Platelet-derived growth factor in human glioma. *GLIA* 15, 257-263.
106. Heldin, C.-H. (1995). Dimerization of cell surface receptors in signal transduction. *Cell* 80, 213-223.
107. Fredholm, B.B., and Heldin, C.-H. (1995). Ny serie: Tillväxtfaktorer. *Läkartidningen* 92, 1454-1457.
108. Fredholm, B.B., and Heldin, C.-H. (1995). Tillväxtfaktorer - mekanismerna klamar. *Läkartidningen* 92, 1459-1467.
109. Heldin, C.-H., and Miyazono, K. (1995). Transforming growth factor- $\beta$ . Intressant kandidat för klinisk användning. *Läkartidningen* 92, 1569-1572.
110. Heldin, C.-H., and Westermark, B. (1996). Role of platelet-derived growth factor *in vivo*. In: *The Molecular and Cellular Biology of Wound Repair* (Clark, R.A.F., ed.), Plenum Press, New York, pp. 249-273.
111. Heldin, C.-H., Östman, A., and Westermark, B. (1996). Platelet-derived growth factor. In: *Growth Factors and Cytokines in Health and Disease* (LeRoith, D., and Bondy, C., eds.), JAI Press Inc., Greenwich, CT, Vol. 1A, pp. 123-145.
112. Yamashita, H., ten Dijke, P., Heldin, C.-H., and Miyazono, K. (1996). Bone morphogenetic protein receptors. *Bone* 19, 569-574.
113. Saras, J., and Heldin, C.-H. (1996). PDZ domain bind carboxy-terminal sequences of target proteins. *Trends. Biol. Sci.* 21, 455-458.
114. Heldin, C.-H. (1996). Protein tyrosine kinase receptors. In: *Cancer Surveys* (Parker, P., and Pawson, T., eds.), Cold Spring Harbor Press, New York, Vol. 27, pp. 7-24.
115. ten Dijke, P., Miyazono, K., and Heldin, C.-H. (1996). Signaling via hetero-oligomeric complexes of type I and type II serine/threonine kinase receptors. *Curr. Opin. Cell Biol.* 8, 139-145.
116. Heldin, C.-H., and Östman, A. (1996). Ligand-induced dimerization of growth factor receptors: variations on the theme. *Cytokines and Growth Factor Reviews* 7, 3-10.
117. Heldin, C.-H., and Purton, M., eds. (1996). Signal Transduction. *Modular Texts in Molecular and Cell Biology*, Chapman and Hall, London.
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Carl-Henrik Heldin

## RESEARCH ARTICLES

1997-05-28

1. Heldin, C.-H., Wasteson, Å., and Westermark, B. (1977). Partial purification and characterization of platelet factors stimulating the multiplication of normal human glial cells. *Exp. Cell Res.* 109, 429-437.
2. Wasteson, Å., Glimelius, B., Busch, C., Westermark, B., Heldin, C.-H., and Norling, B. (1977). Effect of a platelet endoglycosidase on cell surface associated heparan sulphate of human cultured endothelial and glial cells. *Thrombosis Res.* 11, 309-321.
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10. Heldin, C.-H., Westermark, B., and Wasteson, Å. (1981). Demonstration of an antibody against platelet-derived growth factor. *Exp. Cell Res.* 136, 255-261.
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12. Ek, B., Westermark, B., Wasteson, Å., and Heldin, C.-H. (1982). Stimulation of tyrosine-specific phosphorylation by platelet-derived growth factor. *Nature* 295, 419-420.
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Scientific award committees:	<p>Member, Sloan Prize Selection Committee, General Motors Cancer Research Awards, 1988-1989.</p> <p>Vice Chairman, Sloan Prize Selection Committee, General Motors Cancer Research Awards, 1989-1990.</p> <p>Member, Prix Antoine Lacassagne Selection Committee, French National Organisation against Cancer, 1992-1995.</p> <p>Deputy Member, Board of the Göran Gustafsson Foundation, 1994-</p>
Other committees:	<p>Member, Board of the Faculty of Medicine, Uppsala University, 1978-1980.</p> <p>Deputy Member, Board of the Faculty of Medicine, Uppsala University, 1993-</p> <p>Member, Research Committee, Faculty of Medicine, Uppsala University, 1993-</p> <p>Member, Committee to recommend organization of molecular biology at the University of Trondheim, Norway, 1995</p>
Associate editor:	<p>Growth Factors, 1988-</p> <p>Molecular Biology of the Cell (formerly Cell Regulation), 1989-</p> <p>Cancer Research, 1993-</p> <p>Genes to Cells, 1995-</p>
Editorial boards:	<p>European Journal of Biochemistry, 1987-1992</p> <p>In Vitro Cellular and Developmental Biology, 1988-</p> <p>Progress in Growth Factor Research, 1988-1995</p> <p>Biochemistry, 1989-1992</p> <p>Journal of Vascular Medicine and Biology, 1989-</p> <p>International Journal of Cancer, 1989-</p> <p>Oncogenes and Growth Factors Abstracts, 1989-</p> <p>EMBO Journal, 1990-1992</p> <p>European Journal of Cancer, 1990-</p> <p>Trends in Biological Sciences, 1990-</p> <p>Journal of Vascular Research, 1991-</p> <p>Pathogenesis, 1996-</p> <p>Journal of Cellular Physiology, 1996-</p> <p>Cytokine and Growth Factor Reviews, 1996-</p> <p>Journal of Cell Science, 1996-</p>

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	1989	Elected member of European Molecular Biology, Organization
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Awards:	1984	The The Swedberg Prize, from The Swedish Biochemical Society
	1984	King Oscar II:s Prize, from the University of Uppsala (shared with K. Söderhäll)
	1984	The Alvarenga Prize, from the Swedish Medical Society (shared with B. Westernmark)
	1984	The Thureus Prize, from the Royal Society of Sciences (Uppsala)
	1986	Anders Jahre's Medical Prize for Younger Scientists, from the University of Oslo (shared with B. Gustafsson)
	1989	Prix Antoine Lacassagne, from the French National Organization against Cancer (shared with D. Gospodarowicz)
	1989	K. Fernström's Medical Prize for Young Swedish Scientists, from the Medical Faculty of Uppsala University
	1990	The Jubilee Prize, from the Swedish Medical Society (shared with B. Westernmark)
	1992	The EMBO Medal, from the EMBO Council
Thesis adviser:	1993	K. Fernström's Large Medical Prize for Nordic Scientists, from the Medical Faculty of Lund (shared with B. Westernmark)
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Assistant thesis adviser:		Assistant thesis adviser for 10 graduate students: Christer Betsholtz (1986), Monica Nistér (1987), Karin Mellström (1988), Ylva Paulsson (1988), Anders Tingström (1991), Anders Eriksson (1992), Anja Smits (1992), Monica Hermanson (1993), Petra Franzén (1995), Koutaro Yokote (1996).

## CURRICULUM VITAE

97-05-28

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1972-1981 Bachelor of Science (Mathematics 1 1/2 year, Numeric analysis 1/2 year, Psychology 1/2 year, Greek 1/2 year) completed July 28, 1981 (University of Uppsala)

1975-1980 Thesis work at Department of Medical and Physiological Chemistry (University of Uppsala). Dissertation May 10, 1980. "Studies on growth factors for human cultured cells".

Academic positions: 1972-1974 Part time teaching positions at Depts of Anatomy, Medical and Physiological Chemistry, and Physiology (in total 200 hours)

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81.07.01-83.12.31 Cancer Research Scholarship from the Swedish Cancer Society

84.01.01-85.12.31 Senior Scientist of the Swedish Cancer Society

86.01.01-- Director, Ludwig Institute for Cancer Research (Uppsala Branch)

92.08.01-- Professor in Molecular Cell Biology at the Medical Faculty of Uppsala University

C. regulating the growth, differentiation, and functions of endothelial cells, particularly lymphatic endothelia (Preliminary Amendment at p. 7);

D. generating antibodies against the Flt4 ligand (Preliminary Amendment at p. 7);

E. use in an assay to detect the presence of FLT4 receptor tyrosine kinase (see the Preliminary Amendment at p. 19, claim 35); and

F. use in an assay to detect endothelial cell proliferation (*id.*, claim 34).

14. With respect to my conclusions in paragraphs 6-13, above, I believe that the reader of ordinary skill in the field in 1994 who reviewed the 1994 application would have reached the same conclusions: that the inventors had possession of a concept of what is now being claimed in the present application. Stated another way, the priority application reasonably would have conveyed to the skilled artisan that the inventors had possession of the Flt4 ligand invention recited in claims of the 1995 application, of how to purify the ligand, and how to use the ligand.

15. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

June 4, 1999  
Date

Carl-Henrik Heldin  
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application enable the reader to purify the Flt4 ligand by affinity chromatography.

F. The 1994 application teaches to subject the Flt4 ligand material that is eluted from the affinity column to further purification, using ion exchange and reverse-phase high pressure chromatography and SDS-polyacrylamide gel electrophoresis. (See the Preliminary Amendment at p. 15.) While the reader would have been able to perform all three of these conventional techniques, it is clear from the results reported in the 1995 application that sufficiently pure Flt4 ligand is obtained (e.g., sufficiently pure for amino acid sequencing) simply with the affinity purification followed by the SDS-PAGE procedure. (See the 1995 application at Example 5, pp. 17-19.) The ion exchange and reverse-phase chromatography were unnecessary.

Thus, the 1994 application teaches the reader how to purify and isolate an Flt4 ligand. The 1995 application describes results of such a purification, thereby demonstrating that the affinity purification method taught in the 1994 application works successfully.

13. The 1994 application teaches several uses for purified Flt4 ligand. These uses include:

A. Isolating a gene encoding the Flt4 ligand by microsequencing the purified ligand to determine a partial amino acid sequence; generating oligonucleotide probes based on the amino acid sequence (See the Preliminary Amendment, Example 15, p. 15; and Example 12, pp. 11-12); using the oligonucleotides as hybridization probes or PCR primers to isolate a ligand-encoding cDNA clone from a cDNA library generated from PC-3 poly-A RNA (*Id.*, Examples 16 and 17A, p. 16);

B. use in an assay system to screen for inhibitors of Flt4 ligand/Flt4 receptor tyrosine kinase interaction (Preliminary Amendment at pp. 6 and 7);



Sepharose abolished the ability of the conditioned medium to stimulate Flt4 phosphorylation).) This teaching provides direct evidence that the ligand of the invention binds to the extracellular domain of Flt4, and thus that the ligand can be purified using the recombinant Flt4 extracellular domain in affinity chromatography.

D. Example 14 of the 1994 application teaches how to make recombinant Flt4 extracellular domain protein to use in an affinity chromatography matrix to purify the Flt4 ligand. (See, e.g., the Preliminary Amendment at p. 13.) Example 3 of the 1995 application contains a similar teaching.

E. Example 15 of the 1994 application teaches how to purify the Flt4 ligand using affinity chromatography procedures. In one of the procedures, the affinity matrix is Flt4 extracellular domain protein that has been cross-linked to CNBr-activated Sepharose 4B (a commercially available solid support that is useful for generating an affinity matrix). The reader in 1994 would have understood that affinity purification involves contacting the ligand-containing solution with the affinity matrix to permit binding between the ligand and the affinity matrix; washing the affinity matrix to remove unbound impurities; and eluting the ligand with an eluting solution. Typically, all fractions removed from the matrix (wash fractions and elution fractions) are assayed to determine in which fractions the ligand of interest has eluted. Example 15 of the 1994 application teaches to use an Flt4 phosphorylation assay to determine which chromatography fractions contained the Flt4 ligand. (See the Preliminary Amendment at p. 15.) The phosphate buffered saline and phosphate buffer wash solutions that were actually used (see the 1995 application at Example 5, p. 18) are typical wash solutions for a protein affinity chromatography. Moreover, the reader would have known that varying parameters such as ionic strength, pH, and the hydrophilic/hydrophobic character of the eluting solutions are conventional methods for eluting a compound of interest from an affinity chromatography column. Thus, the details in Example 15 of the 1994

deduced amino acid sequence of a precursor of a 23 kD Flt4 ligand taught in the 1994 application. (See, e.g., 1995 application at p. 5, lines 13-20.) Thus, according to the 1995 application, an inherent property of an Flt4 ligand taught in the 1994 application is that the ligand has an amino acid sequence comprising a portion of SEQ ID NO: 33 that is effective to permit binding to Flt4 receptor tyrosine kinase and stimulate phosphorylation thereof. These properties are recited in several claims of the 1995 application other than those specifically discussed above.

12. The 1994 application teaches the reader how to purify and isolate an Flt4 ligand from conditioned medium of a prostatic cell line, using an affinity chromatography method:

A. Example 12 in the 1994 application teaches the reader how to prepare a conditioned medium comprising an Flt4 ligand by culturing the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) for seven days in F12 medium in the absence of serum, and then clarifying the medium by centrifugation. (See the Preliminary amendment at p. 8.) Example 4 in the 1995 application contains a similar teaching.

B. Example 12 in the 1994 application contains experimental data proving that the PC-3 conditioned medium contains a ligand that is capable of stimulating tyrosine phosphorylation of Flt4 receptor tyrosine kinase, in cells expressing Flt4 receptor tyrosine kinase. (See the Preliminary Amendment at pp. 8-11.) Moreover, Example 12 in the 1994 application characterizes the Flt4 ligand as a moiety of at least 10,000 molecular weight, and teaches that the medium can be concentrated with a commercially available Centricon-10 concentrator, in order to increase Flt4 ligand activity. (Preliminary Amendment at p. 11.)

C. Example 12 further teaches that treatment of the concentrated PC-3 conditioned medium with Flt4 extracellular domain fragment coupled to Sepharose beads (a solid support) will remove the Flt4 ligand from the conditioned medium. (See the Preliminary Amendment at p. 11 (pretreatment of the concentrated conditioned medium with Flt4EC-

1995 application at pp. 18-19 (teaching that the Flt4 ligand that was affinity purified from PC-3 medium had an apparent molecular weight of about 23 kD as assessed by SDS-PAGE under reducing conditions).)

B. Claim 15 recites a polypeptide having all of the characteristics described in claim 14 and further recites that the polypeptide comprises "an amino acid sequence set forth in SEQ ID NO: 13." The partial amino acid sequence set forth in SEQ ID NO: 13 of the 1995 application is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from the PC-3 conditioned medium. (See the 1995 application at p. 19, lines 9-19 (teaching that Flt4 ligand that was affinity purified from PC-3 medium had an amino terminal amino acid sequence set forth in SEQ ID NO: 13).)

C. Claim 16 recites a polypeptide having all of the characteristics described in claim 13 and further recites that amino acids 2 through 18 of the polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13. Thus, for the reasons described above with respect to claims 13 and 15 (in Parts A and B), the features recited in claim 16 are inherent properties of an Flt4 ligand that the 1994 application teaches one how to purify from PC-3 conditioned medium.

D. Claim 23 recites a polypeptide having all of the characteristics described in claim 14 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The subject matter of claim 14 is described in the 1994 application. (See paragraph 9, above.) The approximate 23 kD molecular weight further recited in claim 23 is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from a PC-3 conditioned medium, as discussed in Part A above with respect to claim 13.

The foregoing is not intended to constitute a complete list of those claims which recite inherent properties of an Flt4 ligand described in the 1994 application. For example, the 1995 application teaches a cDNA nucleotide sequence and a

Flt4 polypeptide ligand stimulates Flt4 tyrosine phosphorylation in mammalian cells that express Flt4 receptor tyrosine kinase. (See the Preliminary Amendment at pp. 8-11; see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or fragment thereof, which is capable of stimulating the Flt4 receptor....").) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 14 of the 1995 application, at the time that the 1994 application was filed.

10. It is fundamental biochemistry that polypeptides are organic chemical compounds, albeit sometimes large and complex ones. Like all organic chemical compounds, polypeptides may be characterized by any of several inherent physical properties, such as molecular formula and molecular weight. Such physical properties are *inherent* characteristics of organic molecules in that they are intrinsic properties of the molecules. Because polypeptides are themselves composed of covalently-bonded chains of smaller organic moieties called amino acids (of which there are about 20 naturally occurring), it is conventional to express the molecular formula of polypeptides as an amino acid sequence. The amino acid sequence of any polypeptide is an inherent property of that polypeptide.

11. Certain claims in the 1995 application recite subject matter that is described in the 1994 application, and also recite certain inherent properties of that subject matter.

A. For example, claims 13 recites a polypeptide having all of the characteristics described in claim 1 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The subject matter of claim 1 is described in the 1994 application. (See paragraph 6, above.) The approximate 23 kD molecular weight that is recited in claim 13 is an *inherent property* of an Flt4 ligand that the 1994 application teaches one how to purify from the PC-3 conditioned medium. (See the

(See the Preliminary Amendment at p. 19.) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 19 of the 1995 application, at the time that the 1994 application was filed.

8. I conclude that the subject matter of claim 17 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 17 is similar to claim 1 of the 1995 application and additionally recites that the polypeptide is "purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." These additional properties are explicitly described in the 1994 application in Examples 12 and 15: Example 12 teaches that conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) produces a soluble ligand for Flt4 that binds to recombinant Flt4 extracellular domain and that can be purified using the Flt4 EC domain in affinity chromatography (see the Preliminary Amendment at pp. 8-11); Example 15 describes such affinity chromatography. (*Id.* at p. 15.) Thus, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 17 of the 1995 application, at the time that the 1994 application was filed.

9. I conclude that the subject matter of claim 14 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 14 recites, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase." Descriptive support in the 1994 application for "a purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase" is discussed above with respect to claim 1. (See paragraph 6, above.) Example 12 in the 1994 application teaches that the

Therefore, the recitations in claim 1 regarding binding to the Flt4 *extracellular domain* are described in the 1994 application.

D. Claim 31 of the 1994 application recites that the ligand "specifically binds," whereas claim 1 of the 1995 application is directed to "high affinity" binding. However, the reader would have understood that the "ligand" that "specifically binds" to Flt4 receptor was a high affinity binding partner. For example, the teaching in the 1994 application to purify the ligand using the recombinant FLT4 EC domain in affinity chromatography (see, e.g., the Preliminary Amendment at p. 11 and Example 15) apprises the reader that the ligand is thought to be a high affinity ligand.

Thus, I conclude that the subject matter of claim 1 of the 1995 application is described in claim 31, at pp. 11 and 15 of the Preliminary Amendment, and elsewhere in the 1994 application.

7. I conclude that the subject matter of claim 19 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Claim 19 of the 1995 application is directed to the polypeptide having all of the features recited in claim 1 of the 1995 application, and "further comprising a detectable label." Thus, the only aspect of claim 19 not already discussed above (in paragraph 6) is the inclusion of a detectable label. However, claim 33 of the 1994 patent application recites, "The ligand according to claim 31 comprising a label." (See the Preliminary Amendment at p. 18.) Since claims in a patent application define the invention for which patent applicants seek patent protection, it is absolutely clear to the reader from claims 31 and 33 of the 1994 application that the inventors considered an Flt4 ligand which includes a label to be an aspect of their invention. The property of being "detectable" is understood in the art to be inherent in a "label." (The purpose of a label is to provide a means for detecting the substance that carries the label.) Moreover, this understanding is confirmed by claims 34 and 35 of the 1994 application, which are directed to methods which involve "detecting" the labeled ligand.

6. From the facts summarized below, I conclude that the subject matter of claim 1 of the 1995 application is described in the 1994 application in a manner which apprises the reader that the inventors had possession of a concept of what is claimed. Stated another way, the 1994 application reasonably conveys to me that the inventors had possession of the subject matter of claim 1 of the 1995 application, at the time that the 1994 application was filed:

A. Claim 31 of the 1994 application recites, "A ligand which specifically binds to an FLT-4 receptor tyrosine kinase." (See the Preliminary Amendment at p. 18.) Since claims in a patent application define the invention for which patent applicants seek patent protection, it is absolutely clear to me that the inventors considered an Flt4 ligand to be an aspect of their invention.

B. Claim 1 of the 1995 application recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." Thus, whereas claim 31 of the 1994 application was directed to "a ligand," claim 1 of the 1995 application is directed to "a purified and isolated polypeptide." However, the 1994 application clearly states that the ligand of the invention is a purified protein. (See, e.g., the Preliminary Amendment at p. 15 ("The purified biologically active ligand protein . . ."); see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or a fragment thereof, which is capable of stimulating the FLT4 receptor....").) Therefore, the "purified and isolated polypeptide" recitations of claim 1 are described in the 1994 application.

C. Whereas claim 31 of the 1994 application was directed to binding "to an FLT-4 receptor tyrosine kinase," claim 1 of the 1995 application specifies that the ligand binds "to the extracellular domain of Flt4 receptor tyrosine kinase." However, the 1994 application clearly states that the ligand protein binds to the Flt4 *extracellular domain*. (See, e.g., the Preliminary Amendment at p. 11: "The above experiments prove that the ligand binds to the recombinant FLT4 EC [extracellular] domain.")

antibodies reactive with the ligand. I understand that the Ludwig Institute now has an ownership interest in this application.

3. I further understand that, during examination of the 1995 application by the U.S. Patent and Trademark Office (the Patent Office), the examiner has taken the position that U.S. Patent Application Serial No. 08/340,011, filed on 14 November 1994 ("the 1994 application") does not contain a written description of the polypeptide invention that is being claimed in the 1995 application. I have been asked by the Ludwig Institute to review the 1994 and 1995 applications and to provide a factual analysis of whether the 1994 application contains a written description of the invention that is being claimed in the 1995 application.

4. I understand that the claims in a patent application are the portion of a patent application that defines the invention for which patent applicants seek patent protection. I further understand that patent applications are written for the practitioner of ordinary skill in the pertinent scientific field. In the scientific specialties or subdisciplines which fall within the general category of "cellular and molecular biology," the reader of ordinary skill in 1994 and 1995 (hereinafter "the reader") would have had at least a medical or doctorate degree and probably at least some post-doctoral research experience.

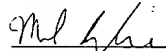
5. To perform this analysis, I have reviewed and understand the contents of the 1994 application. This review included the document titled "Preliminary Amendment" that was filed on 14 November 1994 (hereinafter "the Preliminary Amendment"). I understand that pages 2-19 of the Preliminary Amendment contain text, examples, and claims which are considered part of the 1994 application. I also have reviewed and understand the contents of the 1995 application, including the claims thereof. Exhibit B hereto contains the pending claims of the 1995 application, with claim amendments that the Applicants intend to file with the Patent Office contemporaneously with this declaration.





PATENT  
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:	)	"EXPRESS MAIL"
	)	Mailing label No. EM099827086US
Alitalo et al.	)	
	)	Date of Deposit: June 11, 1997
Serial No.: 08/510,133	)	
	)	I hereby certify that this paper and the documents
Filed: August 1, 1995	)	referred to as enclosed herewith are being
	)	deposited with the United States Postal Service
For: RECEPTOR LIGAND	)	"EXPRESS MAIL POST OFFICE TO ADDRESSEE"
	)	service under 37 CFR §1.10 on the date indicated
Group Art Unit: 1814	)	above and is addressed to the Assistant
	)	Commissioner for Patents,
Examiner: Lathrop, B.	)	Washington, D.C. 20231.
	)	
	)	
	)	Mark Bonadonna

Declaration of Carl-Henrik Heldin  
Pursuant to 37 C.F.R. §1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

RECEIVED  
JUN 16 1997  
GROUP 1800

Sir:

I, Carl-Henrik Heldin, hereby state as follows:

1. I am Director and member of the Uppsala Branch of Growth Regulation of the Ludwig Institute of Cancer Research (the Ludwig Institute) in Uppsala, Sweden. My curriculum vitae is attached hereto as Exhibit A.

2. I understand that on 01 August 1995, Dr. Kari Alitalo and Dr. Vladimir Joukov (as inventors) filed U.S. Patent Application Serial No. 08/510,133 (hereinafter "the 1995 application"), directed to a polypeptide ligand for Flt4 receptor tyrosine kinase; fragments thereof; a polynucleotide encoding the ligand; vectors and host cells comprising the polynucleotide; and

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label.

16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

19. A polypeptide according to claim 1 further comprising a detectable label.

20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. (Amended) A polypeptide according to claim 17 further comprising a detectable label.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

Appendix of claims

1. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an amino acid sequence shown in SEQ ID NO: 33.

8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

9. (Twice amended) A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

12. (Amended) A pharmaceutical composition comprising a polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

13. A polypeptide according to claim 1 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.


Moreover, as explained above, it is within the skill of the art to synthesize deletion mutants of SEQ ID NO: 33 that have been spaced intermittently (e.g., residues 34-180, 34-160, 34-140, 34-120, etc.), rather than synthesize every possible successive deletion mutant (34-180, 34-179, 34-178, 34-177 . . .), to more rapidly identify effective portions for binding Flt4. Furthermore, the skilled artisan is capable of synthesizing and screening several such deletion fragments simultaneously, in parallel experiments. Thus, the examiner's assertions that it is necessary to screen every fragment of SEQ ID NO: 33, that the specification lacks guidance, and that the amount of screening required constitutes undue experimentation is improper. See *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.")

#### VIII. Summary

For the foregoing reasons, the applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification, withdrawal of the notation that no claims are afforded priority to the parent application, and allowance of claims 1-2, 8-9, 12-17, and 19-28.

Respectfully submitted,

June 11, 1997

  
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portion of SEQ ID NO: 33 that is effective for binding, and that portion which is not.<sup>8</sup> An artisan of ordinary skill also understands techniques for accelerating a screening process,<sup>9</sup> and techniques for screening multiple polypeptides *simultaneously*. Thus, the examiner's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

In this regard, the application provides explicit guidance for screening fragments of SEQ ID NO: 33 to determine a portion effective to permit Flt4 binding. Although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the region critical for receptor activation is contained within its first approximately 180 amino acid residues.

By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin, et al., Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues. (Specification, pp. 27-28.)

To determine which fragments contain a sufficient portion of SEQ ID NO: 33 to permit binding, the specification also outlines a specific protocol. The specification teaches one skilled in the art to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. (Specification at, e.g., p. 27, lines 23-29.) These teachings serve to both provide guidance for predicting the portions of

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<sup>8</sup> For example, a determination that a polypeptide comprising residues 34-180 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 181-350 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

<sup>9</sup> For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 34-350, 34-330, 34-310, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (34-350, 34-349, 34-348 . . .), to more rapidly identify effective portions for binding Flt4.

VI. The amendments to claims 21, 22, and 25 place these claims in condition for allowance; and new claim 28 is in condition for allowance.

Claims 21, 22, and 25 have been amended to depend from and further limit claims 16 and 17. New claim 28 is identical to claim 21 and depends from claim 16. Because the subject matter of claims 16 and 17 has been deemed allowable, the amendment of claims 21, 22, and 25 (and addition of claim 28) to depend from claims 16 and 17 also places these claims in condition for allowance. Accordingly, entry of these amendments and allowance of claims 21, 22, 25, and 28 is respectfully requested.

VII. The Patent Office's rejections of claims 1, 8, 9, 13-15, and 19-25 under §112, first paragraph, for lack of enablement improperly ignore both guidance provided in the specification and the skill of those in the art.

In paragraphs 10-13 of the official action, the examiner articulated his basis for maintaining rejections of claims 1, 8, 9, 13-15, and 19-25 under §112, first paragraph, for lack of enablement. The Patent Office admits that fragments of the protein of SEQ ID NO: 33 can be made, but asserts that undue experimentation would be required to screen all fragments of SEQ ID NO: 33 to determine which fragments bind the receptor:

The examiner argues that the claim limitation of binding the receptor must be met by testing all fragments encompassed by the claims, which in this case are not limited in any way.

(official action at p. 7.)<sup>7</sup>

The Patent Office's insistence that it is necessary to test all fragments of SEQ ID NO: 33 ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that

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<sup>7</sup> Claim 8 encompasses only polypeptides which are capable of binding the Flt4 receptor. To the extent that the examiner has interpreted claim 8 (or similarly limited claims) to "encompass" all fragments of SEQ ID NO: 33, the examiner has ignored a limitation of claim 8 and thereby erroneously construed the claim.

subject to review by the Board of Patent Appeals and Interferences. See M.P.E.P. §706.01.

The applicants hereby authorize the commissioner to charge any necessary petition fee associated with this conditional petition to Deposit Account No. 13-2855. This petition has been timely filed within two months of the mailing of the final official action that contains the adverse priority determination at issue.

**IV. The amendments to claim 8 render moot the rejection of claims 8-9 and 19-20.**

In paragraph 9 of the outstanding official action, the examiner rejected claims 8-9 and 20-22 under 35 U.S.C. § 101, asserting that these claims read on a product of nature, because claim 8 fails to recite a "purified and isolated" polypeptide. (Office action at p. 5.)

In response, the applicants have amended claim 8 to recite, "A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Thus, amended claim 8 does not read on a product of nature, rendering the rejection of claim 8 (and claims 9 and 19-20 which depend therefrom) moot. Since this amendment adopts a suggestion of the Patent Office and removes an issue for appeal, entry of the amendment and withdrawal of the rejection is respectfully requested.

**V. The amendments to claims 16 and 17 place these claims in condition for allowance.**

In paragraph 15 of the outstanding action, the Patent Office objected to claims 16 and 17 as being dependent upon a rejected base claim, but indicated that these claims would be allowable if rewritten in independent form. (Office action at p. 11.) In response, the applicants have rewritten claims 16 and 17 in independent form, incorporating all of the limitation of the base claim and any intervening claims. Accordingly, claim 16 and 17 are now in condition for allowance.



priority is when a reference is found with an effective date between the date of the foreign filing and the date of filing in the United States and when an interference situation is under consideration. If at the time of making an action the examiner has found such an intervening reference, he or she simply rejects whatever claims may be considered unpatentable thereover, without paying any attention to the priority date . . . .

(M.P.E.P. (6th Ed., Rev. 2) §201.15.)

The outstanding final action constitutes the first time that the Patent Office has raised its written description objection as a basis for refusing to afford priority to the '011 application.<sup>6</sup> However, there are no prior art rejections based upon intervening references in the outstanding action. Accordingly, under the Patent Office's own procedures, it was inappropriate to consider the merits of the priority claim in the official action.

E. Conditional Petition to Reverse or Withdrawn Adverse Priority Determination.

Should the examiner refuse to reverse or withdraw the adverse priority determination that was made for the first time in the final official action, the applicants hereby petition the commissioner to reverse this determination as improper, or, in the alternative, to withdraw this determination as premature and expunge from the file all mention of this premature determination. The facts in support of reversal of the priority determination are provided in parts A-C, above, and in the Declaration of Dr. Heldin filed herewith. The facts in support of withdrawal of the premature determination are provided in part D, above. In the event of withdrawal, the applicants respectfully submit that all mention of the priority determination in the final official action and this submission by the applicants should be expunged from the file, so as not to taint the file history of the eventual patent in a manner adverse to the applicants.

The priority issue is properly the subject of a petition because the priority determination is not pertinent to any rejection and, therefore, is not

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<sup>6</sup> See note 1, *supra*.

2. The present application is distinguishable from *Fiers* because the invention presently claimed pertains to a purified protein.

In *Fiers*, the Federal Circuit rendered an opinion as to that which is required under §112, first paragraph, for an adequate written description of a DNA invention. The invention claimed in the present application is not a DNA invention;<sup>4</sup> the invention pertains to a purified protein, and the issue concerns whether a priority application contains a sufficient written description of that protein invention. The examiner has failed to articulate why a factual determination in *Fiers* pertaining to a DNA invention is relevant to a factual determination pertaining to a protein invention in the present case.<sup>5</sup> Accordingly, the examiner has failed to meet his burden of establishing a *prima facie* case of lack of written description.

Since the *Fiers* holding is distinguishable on its facts and also was rendered in the context of the state of the art in 1979-81, i.e., about 13-15 years prior to the applicants' 1994 filing date, the *Fiers* opinion fails to support the examiner's written description objection.

- D. The right of priority has no bearing on the patentability of any claim at this time, and therefore, is an inappropriate subject for Patent Office determination.

The Manual of Patent Examining Procedure instructs that a priority determination should be made during *ex parte* prosecution only when an intervening reference is found, upon which a rejection under §102 or §103 would be made:

The only times during *ex parte* prosecution that the examiner considers the merits of an applicant's claim of

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<sup>4</sup> The Patent Office has deemed the DNAs taught in the application to constitute a distinct invention.

<sup>5</sup> In this regard, the Patent Office's attention is directed to *Scripps Clinic v. Genentech Inc.*, 18 U.S.P.Q. 1001 (Fed. Cir. 1991), an opinion issued contemporaneously with the *Amgen* opinion and pertaining to a purified protein invention. The Patent at issue in the *Scripps* case (Reissue Patent No. 32,011) contained claims to a purified protein (Factor VIII:C) and to an affinity method of purifying the protein. No amino acid sequence description was required under §112, first paragraph, for the Patent Office to issue or to reissue this patent.

C. The Patent Office's reliance on the *Fiers* case is improper.

In dismissing the applicants' priority claim on written description grounds, the Patent Office relied upon the Federal Circuit's decision in *Fiers v. Revel*, 25 U.S.P.Q.2d 1601, 1606 (Fed. Cir. 1993). (Official action at pp. 2 and 3.) However, the *Fiers* opinion was rendered on its own distinct set of facts, and was rendered in the context of the state of the art in 1979-81 (i.e., about 13-15 years prior to the applicants' 1994 filing date). Since the issue of written description is factual in nature, *In re Alton, supra*, 37 U.S.P.Q.2d at 1580, the examiner's reliance upon a legal opinion rendered on different facts, and in a much earlier period of the art of molecular biology, is highly suspect from the outset.

1. The present application is distinguishable from the facts of the *Fiers* case because the present application teaches a method of preparing the claimed protein as a natural isolate.

The *Fiers* opinion was based on the premise that a written description of a DNA invention requires the same degree of specificity as a conception of a DNA invention. *Fiers*, 25 U.S.P.Q.2d at 1606. Citing its earlier opinion in *Amgen v. Chugai Pharmaceutical Co.*, 18 U.S.P.Q.2d 1016 (Fed. Cir. 1991), the Court acknowledged that conception of a DNA can occur where one is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. *Fiers*, 25 U.S.P.Q.2d at 1604. In the present case, the 1994 priority application is able to define the Flt4 ligand protein by a method of preparation (e.g., affinity purification using the Flt4 extracellular domain) and by chemical characteristics (e.g., a polypeptide that is capable of stimulating the Flt4 receptor and regulating vascular endothelial cells). Thus, under the standards articulated in the *Fiers* and *Amgen* cases for DNA inventions, the 1994 priority application contains a written description of the Flt4 ligand protein invention claimed herein.

Claim 16 (which depended from claim 13 but has been rewritten in independent form) additionally recites amino terminal amino acid sequence information of the claimed polypeptide. The recited amino acid sequence is an *inherent property* of the Flt4 ligand that the 1994 priority application teaches one how to purify from PC-3 conditioned medium.<sup>3</sup> (See the Heldin declaration at ¶¶ 10 and 11.C.) As such, the inclusion of this property in the present application and in claim 16 does not deprive claim 16 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies, supra*.

New claim 28 is identical to claim 19 except that claim 28 depends from claim 16. Thus, claim 28 finds written description support in the 1994 priority application for the reasons outlined above with respect to claims 16 and 19.

Claim 23 depends from claim 14 and further recites that the polypeptide has "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." This molecular weight limitation is an *inherent property* of an Flt4 ligand that the 1994 priority application teaches one how to purify from the PC-3 conditioned medium, as discussed above with respect to claim 13. (See also the Heldin declaration at ¶¶ 10 and 11.D.) As such, the inclusion of this property in the present application and in claim 23 does not deprive claim 23 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies, supra*.

Moreover, the foregoing is not intended to be a complete list of those claims which find written description support in the specification. See the Heldin declaration at ¶11.)

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<sup>3</sup> Moreover, the 1994 priority application teaches to determine the amino terminal amino acid sequence. (See preliminary amendment to '011 application at p. 15 (Example 15).)

PAGE under reducing conditions. The approximate 23 kD molecular weight is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from the PC-3 conditioned medium. (See the Heldin declaration at ¶¶ 10 and 11.A.) As such, the inclusion of this property in the present application and in claim 13 does not deprive claim 13 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276 (Fed. Cir. 1995) ("A claim in a CIP application is entitled to the filing date of the parent application when the claimed invention is described in the parent specification in a manner that satisfies, inter alia, the description requirement of 35 U.S.C. § 112. . . . [T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); *Ex parte Yamaguchi*, 6 U.S.P.Q.2d 1805, 1807 (PTO Bd. App. 1987) (Claim to compound characterized by a particular x-ray diffraction spectrum has written description support in earlier application that teaches the compound, notwithstanding the absence of any teaching of the x-ray diffraction pattern in the earlier application, because a compound and all of its properties are inseparable); see also *Application of Davies*, 177 U.S.P.Q. 381, 385, 475 F.2d 667, 671-72 (C.C.P.A. 1973) ("[W]e see no impediment to the present applicants' refiling their application and incorporating a discussion of the allegedly unobvious properties [of the invention] while retaining the effective date of the application involved here through §120.")

Claim 15 depends from claim 14 and further recites that the polypeptide "comprises an amino acid sequence set forth in SEQ ID NO: 13." This partial amino acid sequence is an *inherent property* of an Flt4 ligand that the 1994 priority application teaches one how to purify from the PC-3 conditioned medium. (See the present application at p. 19, lines 9-19 (teaching that Flt4 ligand affinity purified from PC-3 medium has an amino terminal amino acid sequence set forth in SEQ ID NO: 13); see also the Heldin declaration at ¶¶ 10 and 11.B.) As such, the inclusion of this property in the present application and in claim 15 does not deprive claim 15 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*; *Ex parte Yamaguchi*; and *Application of Davies*, *supra*.

purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase. These additional limitations find explicit written description support in the 1994 priority application in Examples 12 and 15: Example 12 teaches that conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) produces a soluble ligand for Flt4 that binds to recombinant Flt4 extracellular domain and that can be purified using the Flt4 EC domain in affinity chromatography; Example 15 describes such affinity chromatography. (See the preliminary amendment to the '011 application at pp. 8-11 and 15.) Thus, claim 17 finds written description support in the original claims of the 1994 priority application coupled with the written description provided in Examples 12 and 15. (See the Heldin declaration at ¶ 8.)

Claim 21 as amended is identical to claim 19 except that claim 21 depends from claim 17. Thus, claim 21 finds written description support in the 1994 priority application for the reasons outlined above with respect to claims 17 and 19.

Claim 14 recites "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase." Written description support for "a purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase" is discussed above in relation to claim 1. Example 12 in the 1994 priority application teaches that the Flt4 polypeptide ligand stimulates Flt4 tyrosine phosphorylation in mammalian cells that express Flt4 receptor tyrosine kinase. (See the preliminary amendment to the '011 application at pp. 8-11; see also p. 6 ("In a preferred embodiment of the invention, conditioned medium from the PC-3 cell line comprises a protein or fragment thereof, which is capable of stimulating the Flt4 receptor . . . .") Thus, claim 14 finds written description support in the original claims of the '011 application coupled with the written description provided in Example 12. (See the Heldin declaration at ¶ 9.)

Claims 13 depends from claim 1 and recites that the polypeptide has an apparent molecular weight of approximately 23 kD as assessed by SDS-

tyrosine kinase." By way of comparison, claim 1 of the present application recites, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase."

Claim 1 is unquestionably of similar scope and wording to claim 31 as originally filed. Whereas original claim 31 was directed to "a ligand," claim 1 is directed to "a purified and isolated polypeptide." However, the 1994 priority application clearly states that the ligand of the invention is a purified protein. (See, e.g., preliminary amendment to U.S.S.N. 08/340,011 dated November 14, 1994, at p. 15: "The purified biologically active ligand protein . . . .") Whereas original claim 31 was directed to binding to Flt4 receptor tyrosine kinase, claim 1 clarifies that the ligand binds to *the extracellular domain* of Flt4. However, the 1994 priority application clearly states that the ligand protein binds to the Flt4 *extracellular domain*. (See, e.g., preliminary amendment to U.S.S.N. 08/340,011 dated November 14, 1994, at p. 11: "The above experiments prove that the ligand binds to the recombinant FLT4 EC domain.") Finally, original claim 31 recites that the ligand "specifically binds" whereas claim 1 is directed to "high affinity" binding. However, this difference merely adopts preferred claim language suggested by the examiner in the course of prosecution. Thus, claim 1 is unquestionably of similar scope and wording to an original claim of the '011 patent application. (See the Heldin declaration at ¶ 6.) Accordingly, original claims in the '011 patent application provide written description support for claim 1 of the present patent application. See *In re Koller*, 204 U.S.P.Q. at 706.

Claim 19, which depends from claim 1 and recites that the polypeptide further comprises a detectable label, finds written description support in original claim 33 of the 1994 priority application. See *In re Koller*, 204 U.S.P.Q. at 706; see also the Heldin declaration at ¶ 7.

Claim 17 (which depended from claim 1 but has been rewritten as an independent claim incorporating the limitations of claim 1) is similar to claim 1 and additionally recites that the polypeptide is purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, the cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity

this time, and therefore, is an inappropriate subject for Patent Office determination.

- A. The applicants respectfully request entry into the record and consideration of the expert declaration of Dr. Carl-Henrik Heldin filed herewith.

The Patent Office's reviewing court has explicitly approved of the use of declarations which offer factual evidence to help resolve the issue of "written description" in a patent application, and has held that failure to accord appropriate weight to such declarations constitutes legal error. See *In re Alton*, 37 U.S.P.Q. 1578, 1583 (Fed. Cir. 1996). The applicants have filed herewith the expert declaration of Dr. Carl-Henrik Heldin (the "Heldin declaration") to offer a factual explanation as to why one of ordinary skill in the art would have understood the 1994 priority application to describe the invention presently being claimed. Since the examiner raised the written description issue for the first time in the outstanding final official action,<sup>2</sup> the applicants respectfully request entry of this declaration into the record and consideration thereof with respect to the issue of written description.

- B. The determination that no claims are entitled to priority is legally and factually incorrect.

The law is clear that original claims (i.e., claims contained in the patent application as filed) comply with the written description requirement of §112, because *original claims constitute their own description*. See *In re Koller*, 204 U.S.P.Q. 702, 706 (C.C.P.A. 1980). Moreover, later added claims of similar scope and wording are described by original claims. *Id.*

In the present case, the applicants' 1994 priority application (the '011 application) contained original claims to an Flt4 ligand. For example, original claim 31 recites, "A ligand which specifically binds to an FLT-4 receptor

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<sup>2</sup> The written description issue was not necessitated solely by amendments made by the applicants in response to the first action on the merits, and therefore could have been raised by the Patent Office prior to the issuance of a final action.



New claim 26 depends from claim 8 and further limits claim 8 by adopting a suggestion of the examiner with respect to subject matter that the specification enables. Support for the limitation "within amino acids 1-180 of SEQ ID NO: 33" is found in the specification at p. 28, lines 1-3. New claim 27 further limits claim 26 by reciting a specific amino terminal amino acid residue. The particular amino terminus that is recited in claim 27 corresponds to the amino terminus recited in claim 16. This amino terminus finds written support at p. 19, lines 17-19 of the specification.

II. Restriction Requirement

The applicants have canceled non-elected claims 3-7 and 11 without prejudice.

III. The Applicants respectfully request issuance of an advisory action wherein the Patent Office reverses as incorrect, or withdraws as inappropriate, its determination that no claims in the present application are afforded priority to U.S.S.N. 08/340,011.

In the outstanding official action, the examiner has asserted, for the first time, that no claims in the present application are entitled to priority based upon U.S.S.N. 08/340,011, filed November 14, 1994, *because of an asserted lack of written description* under 35 U.S.C. § 112, first paragraph.<sup>1</sup> For the reasons set forth below, this determination is legally and factually incorrect. Moreover, the right of priority has no bearing on the patentability of any claim at

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<sup>1</sup> In its first official action, the examiner made an initial determination that no claims were afforded priority by the '011 application, because of an *asserted absence of enabling disclosure*. However, that initial determination was made without any consideration of the preliminary amendment portion of U.S.S.N. 08/340,011 (a significant omission, since the '011 application is a Rule 62 continuation-in-part of an earlier application, and the preliminary amendment portion of the '011 application is highly pertinent to the priority issue). In the outstanding final action, the priority determination based on lack of enablement has properly been withdrawn. However, the examiner has, for the first time, raised a new objection to the priority claim, based upon an asserted lack of written description.

C<sup>3</sup>  
ord.

22. (Amended) A pharmaceutical composition comprising a polypeptide according to claim ~~8~~ 17 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

C<sup>4</sup>

25. (Amended) A pharmaceutical composition comprising a polypeptide according to claim ~~14~~ 16 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

-- 26. A polypeptide according to claim 8 wherein said portion of SEQ ID NO: 33 effective to permit such binding is a continuous portion of SEQ ID NO: 33 within amino acids 1-180 of SEQ ID NO: 33.

C<sup>5</sup>

27. A polypeptide according to claim 8 wherein the amino terminus of said portion effective to permit such binding corresponds with position 34 of SEQ ID NO: 33.

28. A polypeptide according to claim 16 further comprising a detectable label. --

#### REMARKS

##### I. History of claims and explanation of amendments.

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In an amendment dated February 10, 1997, the applicants canceled claims 10 and 18, amended claims 1, 8, 9, 14, and 17, and added new claims 20-25. In the present amendment, the applicants cancel claims 3-7 and 11, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28. Thus, upon entry of the foregoing amendments, claims 1-2, 8-9, 12-17, and 19-28 would be pending. A copy of the claims in their amended forms is appended hereto.

The nature of each claim amendment is discussed below in the remarks pertaining to each claim.

#### AMENDMENTS

##### In the specification:

At page 24, line 30, after "Figure 9" please insert -- (SEQ ID NOs: 32 and 33) --.

##### In the claims:

Please cancel claims 3-7 and 11, without prejudice, amend claims 8, 16, 17, 21, 22, and 25, and add new claims 26-28, as shown below.

C' 8. (Three times amended) A purified and isolated polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding.

C<sup>2</sup> 16. (Amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase and having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions, [according to claim 13,] wherein amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13.

17. (Twice amended) A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, [according to claim 1,] said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 7435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

C<sup>3</sup> 21. (Amended) A polypeptide according to claim [8] 17 further comprising a detectable label.



Final Review  
BOX AF

Response Under  
37 CFR 1.116 - Expedited  
Procedure Examining  
Group 1814

PATENT  
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Alitalo *et al.*

Serial No: 08/510,133

Filed: August 1, 1995

Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

EXPRESS MAIL LABEL NO:

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Date of Deposit: June 11, 1997

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Assistant Commissioner for Patents,  
Washington, D.C. 20231

Mark Bonadonna

AMENDMENT AFTER FINAL ACTION

and

CONDITIONAL PETITION TO REVERSE OR WITHDRAWN ADVERSE PRIORITY  
DETERMINATION PURSUANT TO  
37 C.F.R. §1.181

BOX AF  
Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:


In an official action mailed April 11, 1997, the examiner finally  
rejected claims 1, 8, 9, 13-15, and 19-25 variously under 35 U.S.C. §§ 101 and  
112, first paragraph. Claims 2 and 12 were allowed, and claims 16 and 17  
were objected to as being dependent upon a rejected base claim, but were  
otherwise deemed allowable. The applicants respectfully request  
reconsideration in light of the following amendments and remarks.

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The examiner will attempt to respond to voice messages within 24 hours. Alternately, the examiner's supervisor, Vasu Jagannathan, can be reached at (703) 306-2777. The FAX number for Art Unit 1801 is (703) 305-7401.

5 An inquiry of a general nature relating to the status of this application should be directed to the Group 1800 receptionist whose telephone number is (703) 308-0196.

  
Brian K. Lathrop, Ph.D.

4/8/97

VASU S. JAGANNATHAN  
PRIMARY EXAMINER  
GROUP 1800

Serial Number: 08/510133  
Art Unit: 1801

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15. Claims 16 and 17 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

5 *Conclusion*

16. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for response to this final action is set to expire THREE  
10 MONTHS from the date of this action. In the event a first response is filed within TWO  
MONTHS of the mailing date of this final action and the advisory action is not mailed until after  
the end of the THREE-MONTH shortened statutory period, then the shortened statutory period  
will expire on the date the advisory action is mailed, and any extension fee pursuant to 37  
CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the  
15 statutory period for response expire later than SIX MONTHS from the date of this final action.

17. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop, whose phone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

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For the reasons set forth, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

13. Applicant's arguments filed 2/13/97 have been fully considered but they are not persuasive.
- 5 Applicants argue that a considerable amount of experimentation is tolerated if that experimentation is routine in nature. While the quantity of experimentation is not *in itself* sufficient for determining undue experimentation, the vast amount of experimentation required to test all the encompassed fragments is still correctly considered as a factor as noted by the examiner in the overall determination of whether the experimentation required to make the
- 10 invention is undue. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988). Other remarks of applicants are made in regard to amendments to the claims and are fully considered in the rejection set forth above.

*Allowable Subject Matter*

- 15 14. Claims 2 and 12 are allowed. Applicants comments concerning WO 95/24473 are noted. By clarification, the examiner notes that this publication discloses a **polypeptide** with greater than 99% sequence identity to the instantly claimed protein of SEQ ID NO:33. Applicants correctly note that it was the polynucleotide disclosed in WO 95/24473 that was obtained from ESTs.

subject matter indicated as enabled; i.e., the subject matter of claims 16 and 17. There is no guidance to predict *a priori* whether any protein would bind the receptor *without some information on the structure of the protein*, and this information was simply not available for all the proteins encompassed by the claims at the time of the invention. The scope of the required enablement varies inversely with the degree of predictability involved, and in applications directed to inventions in arts where the results are unpredictable, the disclosure of a single species usually does not provide an adequate basis to support generic claims. MPEP 2164.03 citing *In re Soll*, 97 F.2d 623, 38 USPQ 189 (CCPA 1938) and *In re Fisher*, 427 F.2d 833, 166 USPQ 18 (CCPA 1970).

10           The scope of claims 15 and 24 encompasses only those proteins comprising SEQ ID NO:13 or fragments of SEQ ID NO:33 capable of stimulating Flt4 receptor tyrosine kinase activity. As set forth above in regard to the activity of fragments of the protein of SEQ ID NO:33, the specification predicts without direct evidence that the first 180 amino acids of SEQ ID NO:33 will be sufficient for biological activity (p. 28, lines 1-3). Beyond this guidance, the  
15           specification provides no guidance to select fragments of the protein of SEQ ID NO:33 that are able to bind the Flt4 receptor. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation  
20           required to test all the encompassed fragments is one factor to be considered in the overall determination of whether the experimentation required to make the invention is undue.



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vast amount of experimentation required to test all the encompassed fragments is still correctly considered as a factor as noted by the examiner in the overall determination of whether the experimentation required to make the invention is undue. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

5

12. Claims 1, 13-15, 19, and 23-25 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the scope of claim 16 or claim 17, does not reasonably provide enablement commensurate with the scope of the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

10

The scope of claims 1, 13, 14, 19, 23, and 25 encompasses polypeptides from any source that bind with high affinity to the Flt4 receptor or stimulate its tyrosine kinase activity. Claims 13 and 23 require that these polypeptides have an apparent molecular weight of 23 kD on SDS-PAGE under reducing conditions. Making the invention requires testing all tissues from all known species, because neither the source nor the structure of the protein is recited in the instant claims. The teaching of the specification that the peptide is 23 kD provides no real guidance to make the invention commensurate with the scope of the claims, because many proteins are about this size, and the skilled artisan would be further burdened with determining the relative molecular weight of every protein from every tissue from every species known to meet the claim limitation.

15

20 There is no guidance provided by the specification to select those encompassed polypeptides that bind the Flt4 receptor with high affinity with the exception of those teachings which support the

activity of binding the Flt4 receptor given the guidance provided in the specification. Applicants argue that claim 8 encompasses only peptides capable of binding the receptor, and therefore is more limited in scope than the examiner contends. The examiner argues that the claim limitation of binding the receptor must be met by testing all fragments encompassed by the claims, which in this case are *not* limited in any way. Applicants argue that the guidance of the specification directs the artisan to try the amino terminal 130 amino acids, but they do not *claim* fragments comprising the first 180 amino acids of SEQ ID NO:33. Without this claim limitation, should the skilled artisan be guided by the fact that the only biologically active fragment shown is 23 kD in apparent molecular weight with an unknown degree of post-translational modification, or should they be guided by the supposed biological criticality of the amino terminal 180 residues?

Applicants assert that remarks made by the examiner support the argument that there is enabled subject matter. The examiner agrees entirely but notes that the instant claims are not limited in scope to what the examiner indicated as enabled in his action mailed 9/10/96. Applicants argue that the assertion of unpredictability ignores the guidance in the specification, and that the alleged vast amount of experimentation required to make the experiment does not take the routine nature or the level of the skilled artisan into account. Unpredictability is generated by the uncertain result on biological activity when protein structure is altered, which may only be alleviated by providing some guidance as to which structures are necessary and sufficient for biological activity. The examiner has agreed that applicant has provided such guidance in the specification where the specification teaches the criticality of the first 180 residues for biological activity. While the quantity of experimentation is not *in itself* sufficient for determining undue experimentation, the

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NO:33 that is produced by 293-EBNA cells has biological activity, but that the protein of SEQ ID NO:33 is predicted to be about 35.7 kD in molecular weight. The specification does not teach which amino acids are present in the 23 kD fragment, nor does it teach whether post-translational modifications such as glycosylation may have changed the apparent molecular weight. The specification predicts without direct evidence that the first 180 amino acids of SEQ ID NO:33 will be sufficient for biological activity (p. 28, lines 1-3). Beyond this guidance, the specification provides no guidance to select fragments of the protein of SEQ ID NO:33 that are able to bind the Flt4 receptor. Without additional structural information on the ligand, the skilled artisan cannot predict which additional fragments of the protein of SEQ ID NO:33 might bind the receptor. Where the art is unpredictable, as in the case of physiological activity, more guidance is required. *In re Fisher*, 166 USPQ 18 (CCPA 1970). The vast amount of experimentation required to test all the encompassed fragments is one factor to be considered in the overall determination of whether the experimentation required to make the invention is undue. For the reasons set forth above, undue experimentation would be required to make the invention commensurate with the scope of the claims. *In re Wands*, 8 USPQ2d 1400, 1404 (CAFC 1988).

11. Applicant's arguments filed 2/13/97, Paper No. 15, have been fully considered but they are not persuasive. Applicants argue that the skilled artisan can make any fragment of the protein of SEQ ID NO:33 and that the determination of whether these fragments can bind the receptor requires routine experimentation. The examiner agrees that while fragments could be made, undue experimentation would be required to make those fragments with the claimed biological

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Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

9. Claim 8 and dependent claims 9 and 20-22 are rejected under 35 U.S.C. 101 because the  
5 claimed invention is directed to non-statutory subject matter. Claim 8 as amended no longer  
recites a purified and isolated polypeptide and hence reads on a product of nature, which is non-  
statutory subject matter.

*Claim Rejections - 35 USC § 112*

- 10 10. Claims 8, 9, and 20-22 are rejected under 35 U.S.C. 112, first paragraph, because the  
specification, while being enabling for a portion of SEQ ID NO:33 capable of binding to an Flt4  
receptor tyrosine kinase comprising about the amino terminal 180 amino acids, does not  
reasonably provide enablement commensurate with the scope of the claims. The specification  
does not enable any person skilled in the art to which it pertains, or with which it is most nearly  
15 connected, to make the invention commensurate in scope with these claims.

Making the invention requires a portion of the protein of SEQ ID NO:33 effective to  
permit binding to the Flt4 receptor. Claim 9 recites that this fragment must have an apparent  
molecular weight of 23 kD determined by SDS-PAGE under reducing conditions. Claim 20  
further requires that the fragment bind with high affinity and stimulate Flt4 receptor  
20 phosphorylation. The specification guides the selection of Flt4 ligands comprising a portion of  
SEQ ID NO:33 capable of binding the Flt4 receptor and stimulating its tyrosine kinase activity at  
Example 11. The specification teaches that a 23 kD polypeptide from the protein of SEQ ID

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issue process. Applicants correctly note that formal drawings are not required at this stage in prosecution.

*Withdrawn Objections and Rejections*

5 7. The objections to claims 14 and 17 are withdrawn in view of applicants' arguments and amendments. The remarks regarding the enabling disclosure are withdrawn from the consideration of whether the claims should be objected to under 37 CFR 1.75 as not pertinent to this Rule. Any remarks about the conformity of the claims to the enablement requirement will be made as appropriate in the context of 35 USC 112, first paragraph, as set forth below: The  
10 rejection of claims 1 and 8 and dependent claims 9 and 13-19 under 35 USC 112, second paragraph, is withdrawn in view of applicants' arguments. The rejections of claim 10 under 35 USC 112, second paragraph, are rendered moot by the cancellation of claim 10. The rejection of claims 8 and 9 under 35 USC 112, second paragraph, is withdrawn in view of applicants' amendment. The rejection of claim 18 under 35 USC 112, first paragraph, is rendered moot by  
15 the cancellation of the claim. The rejection of claim 1 under 35 USC 102(a) as being anticipated by Pajusola et al. is withdrawn in view of applicants' amendment. The rejection of claim 18 under 35 USC 102(b) as being anticipated by Sitaras et al. is rendered moot by the cancellation of claim 18.

*Claim Rejections - 35 USC § 101*

20

8. 35 U.S.C. 101 reads as follows:

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decision in *Fiers v. Revel*, 25 USPQ2d 1601, 1606 (CA FC 1993) as applied to the claimed ligand. The description in '011 of a conditioned medium containing a ligand or ligands that activate the Flt4 receptor and a potential method for isolating these ligands does not satisfy the written description requirement of 35 USC 112, because '011 does not describe the ligand itself,  
5 nor does it demonstrate that the disclosed method would actually produce the claimed ligand. '011 therefore does not demonstrate that the inventor had possession of claimed ligand at the time it was filed. No claims are afforded priority to application Serial No. 08/340011 for the reasons set forth.

- 10 4. The examiner notes the mention of Finnish application Serial No. 950624 in the Declaration filed 2/27/97 without the claim of priority to this application under 35 USC 119.

#### *Drawings*

- 15 5. The corrected or substitute drawings were received on 2/13/97. These drawings are acceptable.

6. Since allowable subject matter has been indicated, applicant is encouraged to submit formal drawings in response to this Office action. The early submission of formal drawings will permit the Office to review the drawings for acceptability and to resolve any informalities  
20 remaining therein before the application is passed to issue. This will avoid possible delays in the

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## DETAILED ACTION

### *Election/Restriction and Disposition of Claims*

1. Applicants remarks concerning the finality of the requirement for restriction are noted.
- 5 Applicants argue that 35 USC 121 should be interpreted as requiring the inventions to be both "independent" and "distinct" to be properly restricted. The restriction requirement is final as set forth in Paper No. 11, mailed 9/10/96. Applicants are respectfully referred to the court's decision in *In re Lee*, 199 USPQ 108, 109 (ComrPats 1978).
- 10 2. Requirement of cancellation of non-elected claims is premature as noted by applicant and is withdrawn. Claims 1-9, 11-17, and 19-25 are pending, with claims 3-7 and 11 withdrawn from consideration as to a non-elected invention.

### *Priority*

- 15 3. In support of their position that some claims may receive the benefit of priority under 35 USC 120, applicants point out that the examiner missed support for a Flt4 ligand in a preliminary amendment in application Serial No. 08/340011 ('011). The preliminary amendment in '011 describes conditioned medium from PC-3 cell cultures that comprises a soluble ligand for the Flt4 receptor (p. 11, last paragraph). '011 does not describe the ligand, but rather describes the  
20 induction of receptor phosphorylation in response to a ligand and states that ligands may be purified from the conditioned medium (p. 6, penultimate paragraph). The examiner relies on the



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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
33/310,133	08/01/95	ALITALO	K 28113/32863

13M2/0411  
MARSHALL O'TOOLE GERSTEIN MURRAY  
AND BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

EXAMINER	
LATHROP, B	
ART UNIT	PAPER NUMBER
1801	16 ✓
DATE MAILED: 04/11/97	

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☒ Responsive to communication(s) filed on 4/13/97 (R-L-E)
- ☒ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-9, 11-17, 18-25 is/are pending in the application.
- ☐ Of the above claim(s) 2-7, 11 is/are withdrawn from consideration.
- ☒ Claim(s) 1-2 is/are allowed.
- ☒ Claim(s) 1, 9, 2, 13-15, 19-25 is/are rejected.
- ☒ Claim(s) 16, 17 is/are objected to.
- ☐ Claim(s) \_\_\_\_\_ are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.
- ☐ This proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been received.
- ☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).
- \*Certified copies not received: \_\_\_\_\_

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☐ Notice of Reference Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 13
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/FI 96/00427

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9524473	14-09-95	AU-A- 7394194 EP-A- 0751992 ZA-A- 9403464	25-09-95 08-01-97 20-11-95
WO-A-9533772	14-12-95	AU-A- 2738895	04-01-96

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/FI 96/00427

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ONCOGENE, vol. 9, 1994, pages 3545-3555, XP002022270 K. PAJUSOLA ET AL.: "Signalling properties of FLT4, a proteolytically processed receptor tyrosine kinase related to two VEGF receptors" cited in the application see the whole document, in particular page 3553. ---	1-3,5, 18, 29-31,34
X	ONCOGENE, vol. 8, 1993, pages 2931-2937, XP002022271 K. PAJUSOLA ET AL.: "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts" cited in the application see the whole document, in particular page 2936. ---	1-3,5, 18, 29-31,34
X	EMBL Database entry HS991157, accession no. M07991, 2 July 1995; HILLIER L. ET AL. "The WashU-Merck EST Project" XP002022299 see the sequence. ---	37,38
P,X	WO,A,95 24473 (HUMAN GENOME SCIENCES, INC.) 14 September 1995 see the whole document, especially Figure 1 and the claims. ---	1-44
P,X	WO,A,95 33772 (K. ALITALO ET AL.) 14 December 1995  see Examples 8 and 9 and the claims. ---	1-3,5, 18,22, 29-31,34
P,X	EMBO J., vol. 15, 1996, pages 290-298, XP002022272 V. JOUKOV ET AL.: "A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGF-2) receptor tyrosine kinase" cited in the application see the whole document. -----	1-44

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/FI 96/00427

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/12 C07K14/52 C07K19/00 C07K16/24 A61K38/19  
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12N C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ONCOGENE, vol. 10, 1995, pages 973-984, XP002022269 J.-P. BORG ET AL.: "Biochemical characterization of two isoforms of FLT4, a VEGF receptor-related tyrosine kinase" see the whole document, in particular the abstract and materials and methods sections. --- -/-	1-3,5, 18, 29-31,34



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

### \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*A\* document member of the same patent family

Date of the actual completion of the international search

29 January 1997

Date of mailing of the international search report

05.03.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2  
NL - 2250 HAV Rijswijk  
Tel. (+ 31-70) 340-2040, Tx. 31 631 cpo nl,  
Fax (+ 31-70) 340-3016

Authorized officer

Yeats, S

## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

RECEIVED  
MAY 6 1996  
GROUP 1800

Applicant's or agent's file reference <b>28999</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/FI 96/00427</b>	International filing date(day/month/year) <b>01/03/1996</b>	(Earliest) Priority Date (day/month/year) <b>01/08/1995</b>
Applicant <b>HELSINKI UNIVERSITY LICENSING LTD OY et al.</b>		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (see Box I).
2. ☐ Unity of invention is lacking (see Box II).
3. ☐ The international application contains disclosure of a nucleotide and/or amine acid sequence listing and the international search was carried out on the basis of the sequence listing.
  - ☐ filed with the international application.
  - ☐ furnished by the applicant separately from the international application,
    - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
  - ☐ Transcribed by this Authority
4. With regard to the title, ☒ the text is approved as submitted by the applicant.  
☐ the text has been established by this Authority to read as follows:
5. With regard to the abstract, ☒ the text is approved as submitted by the applicant.  
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:  
Figure No. \_\_\_\_\_ ☐ as suggested by the applicant. ☒ None of the figures.  
☐ because the applicant failed to suggest a figure.  
☐ because this figure better characterizes the invention.



SHEET 1 of 1

Form 9 (10-1449) (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28967/32863	Serial No. 08/510,133
<b>INFORMATION DISCLOSURE STATEMENT</b>		Applicant Alitalo and Joukov	
(Use several sheets if necessary)		Filing Date 08/01/95	Group 1814

19

U.S. PATENT DOCUMENTS							
*Examiner Initials	Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate	


FOREIGN PATENT DOCUMENTS								
*Examiner Initials	Document Number	Publication Date	Country	Class	Subclass	Translation		
						Yes	No	
	B6	WO 95/33772	12/14/95	PCT				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
C111	Hillier <i>et al.</i> , "The WashU-Merch EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.	

EXAMINER	DATE CONSIDERED
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

# FILE COPY

28 at 1000  
SHEET 1 of 1

Form PTO-1449 (Rev. 10-1993)  <b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)	U.S. Department of Commerce Patent and Trademark Office		Atty. Docket No. 28967/32863	Serial No. 08/510,133
	Applicant Alitalo and Joukov			
	Filing Date 08/01/95		Group 1814	

8

U.S. PATENT DOCUMENTS							
*Examiner Initials		Document Number	Issue Date	Name	Class	Subclass	Filing Date If Appropriate

FOREIGN PATENT DOCUMENTS								
*Examiner Initials		Document Number	Publication Date	Country	Class	Subclass	Translation	
							Yes	No
ca	B6	WO 95/33772	12/14/95	PCT				

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
ca	C111	Hillier <i>et al.</i> , "The WashU-Merch-EST Project," EMBL Database entry HS991157, accession no. H07991, July 2, 1995.

EXAMINER - <i>C. Saoud</i>	DATE CONSIDERED <i>9/27/00</i>
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

This Information Disclosure Statement is not intended to be an admission that other relevant art does not exist, or that any of the information disclosed herein constitutes prior art under 35 U.S.C. §102 or §103.

Pursuant to 37 C.F.R. §1.97(e)(1), the Applicants certify that each document itemized on the attached form PTO-1449 was cited in a communication (an ISR) from a foreign patent office (the European Patent Office) in a counterpart foreign (PCT) application, not more than three months prior to the filing of this statement. Accordingly, pursuant to 37 C.F.R. §1.97(c)(2), the information disclosed herein should be considered by the Patent Office without payment of any fee.

However, the Patent Office is hereby authorized to charge any fees due in connection with this paper to Deposit Account No. 13-2855. A duplicate copy of this document is enclosed herewith.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: April 10, 1997

By: David A. Gass  
David A. Gass  
Registration No.: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300



PATENT APPLICATION  
28967/32863

GROUP 1800

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: Alitalo, Kari	)	I hereby certify that this paper and
	)	the documents referred to as enclosed
and Joukov, Vladimir	)	herewith are being deposited with the
	)	United States Postal Service as First
Serial No.: 08/510,133	)	Class Mail, postage prepaid, in an
	)	envelope addressed to: Assistant
Filed: August 1, 1995	)	Commissioner for Patents,
	)	Washington, DC 20231, on this date:
For: "Receptor Ligand"	)	Date: <u>April 10, 1997</u>
	)	<u>David A. Gass</u>
Group Art Unit: 1814	)	David A. Gass
	)	Reg. No.: 38,153
Examiner: B.K. Lathrop, Ph.D.	)	Attorney for Applicants

**INFORMATION DISCLOSURE STATEMENT  
PURSUANT TO 37 C.F.R. §§ 1.56, 1.97, AND 1.98**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the Applicants wish to call to the attention of the Examiner the enclosed documents, as itemized on Form PTO-1449, which may be considered material to the examination of the above-identified patent application. A copy of each itemized document is enclosed herewith. All of the documents were identified in an International Search Report (ISR, copy enclosed herewith) in a related PCT patent application. Documents identified in the ISR that are not itemized on the attached Form PTO-1449 have already been made of record by the Patent Office or the applicants.





If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27).

NAME: Ludwig Institute for Cancer Research  
ADDRESS: 1345 Avenue of the Americas, New York, NY 10105  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☒ NONPROFIT ORGANIZATION

NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Heikki Lampi

TITLE OF PERSON OTHER THAN OWNER: President

ADDRESS OF PERSON SIGNING: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

SIGNATURE: \_\_\_\_\_

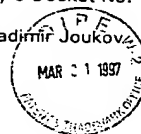
Date

22. Feb. 1997

**PATENT**

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov  
Serial or Patent No: 08/510,133  
Filed or Issued: August 1, 1995  
For: Receptor Ligand



**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(c)) -- SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☐ The owner of the small business concern identified below:
- ☒ An official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Helsinki University Licensing, Ltd.

ADDRESS OF BUSINESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.12, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to, and remain with, the small business concern identified above with regard to the invention, entitled Receptor Ligand, by inventor(s) Kari Alitalo and Vladimir Joukov, described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Edward A McDermott

TITLE IN ORGANIZATION: President

ADDRESS OF PERSON SIGNING: 1345 Avenue of the Americas, New York  
NY 10105

SIGNATURE: *Edward A. McDermott*

Date: March 2, 1997

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled RECEPTOR LIGAND, by inventor(s) Kari Alitalo and Vladimir Joukov

described in

- ☐ The specification filed herewith.
- ☒ Application Serial No. 08/510,133, filed August 1, 1995.
- ☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization regarding the above-identified invention. If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights in the invention is listed below and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

FULL NAME: Helsinki University Licensing, Ltd.  
ADDRESS: Viikinkaari 8 A, FIN-00710 Helsinki, Finland

☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME: \_\_\_\_\_  
ADDRESS: \_\_\_\_\_

☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

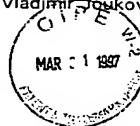
I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that

**PATENT**

Attorney's Docket No: 28967/32863

Applicant or Patentee: Kari Alitalo and Vladimir Joukov  
Serial or Patent No: 08/510,133  
Filed or Issued: August 1, 1995  
For: Receptor Ligand



**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) and 1.27(d)) -- NONPROFIT ORGANIZATION**

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: Ludwig Institute for Cancer Research

ADDRESS OF ORGANIZATION: 1345 Avenue of the Americas  
New York, NY 10105

**TYPE OF ORGANIZATION**

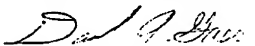
- ☐ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
- ☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c)(3))
- ☐ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_)  
(CITATION OF STATUTE \_\_\_\_\_)
- ☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501 (a) and 501 (c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
- ☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA  
(NAME OF STATE \_\_\_\_\_)  
(CITATION OF STATUTE \_\_\_\_\_)

the co-owners of the patent application and of the invention described therein.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Dated: *March 27, 1997*



David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, IL 60606-6402  
Telephone: (312) 474-6300



9012

PATENT  
28967/32863

1804

447

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of: )

Alitalo et al. )

Serial No.: 08/510,133 )

Filed: August 1, 1995 )

For: RECEPTOR LIGAND )

Group Art Unit: 1814 )

Examiner: Lathrop, B. )

I hereby certify that this paper  
is being deposited with the  
United States Postal Service as  
first class mail, postage  
prepaid, in an envelope  
addressed to: Assistant  
Commissioner for Patents  
Washington, D.C. 20231, on this  
date:

Date: March 27 1997

David A. Gass  
David A. Gass  
Registration No. 38,153  
Attorney for Applicant(s)

## STATEMENT CLAIMING SMALL ENTITY STATUS

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Accounting Division  
Office of Finance

Sir:

The Applicants hereby claim small entity status in  
the above-identified matter for purposes of paying fees,  
pursuant to 37 C.F.R. 1.27, based on the following documents  
transmitted herewith:

- 1) Verified Statement (Declaration) Claiming  
Small Entity Status (37 CFR 1.9(f) and  
1.27(c)) -- Small Business Concern; and
- 2) Verified Statement (Declaration) Claiming Small  
Entity Status (37 CFR 1.9(f) and 1.27(d)) --  
Nonprofit Organization.

The Verified Statements are signed by Heikki Lampi, on behalf  
of Helsinki University Licensing, Ltd., and Edward A.  
McDermott, on behalf of Ludwig Institute for Cancer Research,



The failure of the HGS application to identify a receptor through which the putative "VEGF2 polypeptide" mediates any putative biological activity is significant, and clarification for the record is respectfully requested.

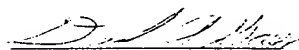
**XVI. Summary**

For the foregoing reasons, the Applicants respectfully request reconsideration, withdrawal of all claim rejections and objections to the specification and claims, and allowance of claims 1-9, 11-17, and 19-25.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Dated: February 10, 1997



David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

The Applicants respectfully submit that the foregoing characterization of the HGS publication (PCT publication WO95/24473) includes conclusions made *after reading the present application*, and that the HGS publication discloses less than the Examiner has indicated.

The Examiner stated that "Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand." This characterization overstates the teachings in the HGS publication. Importantly, the HGS publication fails to disclose or suggest that any polypeptide or other molecule is an *Flt4 receptor ligand*. It is the present application that discloses that a polypeptide is a ligand for Flt4. The Examiner has concluded, based upon the similarity of an HGS sequence to a sequence disclosed by the present applicants, that the HGS sequence is that of an Flt4 ligand. However, the Examiner's familiarity with the present application was required for this conclusion. It is the present application, and not the HGS publication, which identifies an Flt4 receptor tyrosine kinase ligand.<sup>5</sup>

The Examiner also stated, "The ligand of Human Genome Sciences, Inc. was purified using expressed sequence tags . . . ." Apparently, the Examiner intended to state that the nucleotide sequence taught in the HGS publication was obtained using expressed sequence tags . . . . The HGS publication does not disclose "purification" of a "ligand." At best, Example 2 in the HGS publication purports to disclose *in vitro* transcription and translation of three polynucleotides and analysis of the reaction products on an SDS-PAGE gel. The HGS publication states that translated products with estimated molecular weights of "38-40 dk" and "36-38 kd" were observed on the gel.

---

<sup>5</sup> As discussed in detail in preceding sections, the guidance provided in the present application that an *Flt4 ligand* has been identified has important legal ramifications under §112, first paragraph. For example, one would not be motivated to perform *in vitro* screening assays on fragments or variants of a protein, using Flt4 (or an extracellular fragment thereof), if one is unaware that the protein itself is an Flt4 ligand.

Finally, as stated above, claim 1 is entitled to a priority date of November 14, 1994, the filing date of the priority document. Accordingly, Pajusola *et al.*, stated by the Patent Office to have been published in December, 1994, does not constitute prior art.

For all of these reasons, the rejection under §102(a) must be withdrawn.

XIV. The rejection under §102(b) has been rendered moot.

In paragraph 20 of the Office action, the Patent Office rejected claim 18 under §102(b), asserting that the claim is anticipated by Sitaras *et al.*:

Claim 18 is rejected under 35 U.S.C. §102(b) as being anticipated by Sitaras *et al.*

Sitaras *et al.*, the whole document, teach a conditioned medium, thus anticipating the claimed invention. Because this conditioned medium is from PC-3 prostatic adenocarcinoma cells, it has the inherent property of comprising the polypeptide of claim 1.

(Office action at p. 11.)

The Applicants have canceled claim 18 herein, rendering this rejection moot.

XV. Applicants comments concerning the Examiner's statement of reasons for the indication of allowable subject matter.

In paragraph 22 of the Office action, the Examiner stated certain reasons for the indication of allowable subject matter in the present application. Included in the statement of reasons were characterizations of a Human Genome Sciences (HGS) PCT publication that does not constitute prior art:

Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand, but the date of publication does not antecede the filing date of the instant application. The ligand of Human Genome Sciences, Inc. was purified using expressed sequence tags without identification as encoding a vascular endothelial growth factor; therefore, one of ordinary skill in the art at the time of the invention would not have been motivated to use these art-known sequences to arrive at the instant invention.

(Office action at p. 12.)

property of the fusion protein is the Flt4 receptor tyrosine kinase. Pajusola et al. thus anticipate claim 1.

(Office action at p. 11.)

Claim 1 has been amended herein to recite, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase."

Pajusola et al., *Oncogene*, 9:3545-3555 (1994) discloses that colony stimulating factor-1 (CSF-1) specifically activated a fusion protein comprising the ligand binding extracellular domain of the colony stimulating factor-1 receptor (CSF-1R) fused to the transmembrane and cytoplasmic domains of an Flt4 receptor. (See, e.g., p. 3549, col. 2.) The fusion protein was expressed in specified cell lines. The transgenic cells expressing the fusion protein were stimulated using CSF-1.

One skilled in the art understands that CSF-1 binds to the CSF-1 receptor, and more particularly to the extracellular domain of the CSF-1 receptor. The receptor chimera in Pajusola et al. comprised the extracellular domain of the CSF-1 receptor and the transmembrane and intracellular domains of the Flt4 receptor. Thus, it is apparent to one skilled in the art that when CSF-1 is shown to bind this CSF-1R/Flt4 chimera in transfected cells, the binding occurs between the CSF-1 and the CSF-1 receptor extracellular domain portion of the chimera. Thus, Pajusola et al. discloses a purified polypeptide which binds to a chimeric polypeptide comprising the extracellular domain of CSF-1R. Pajusola et al. fails to disclose or suggest a purified polypeptide that binds to Flt4. Accordingly, the rejection of claim 1, which requires a purified and isolated polypeptide capable of binding with high affinity to Flt4 receptor tyrosine kinase, must be withdrawn.

Moreover, amended claim 1 requires a purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase. The chimeric receptor described in Pajusola et al. does not include the extracellular domain of Flt4. For this additional reason, the anticipation rejection of claim 1, based upon Pajusola et al., must be withdrawn.

Similarly, claim 14 has been amended to recite, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase. Thus, amended claim 14 requires more than mere "relatively weak" interaction with Flt4; amended claim 14 requires interaction that stimulates Flt4 receptor phosphorylation, as exemplified in the application.

Claim 17 has been amended to recite, "A purified and isolated polypeptide according to claim 1, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line . . . using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase." Amended claim 17 also is commensurate in scope with the teachings in the application. Even if, as the Patent Office asserts, many different growth factors were found in PC-3 conditioned medium, one would not expect the many different growth factors to bind Flt4 with sufficient affinity to permit affinity purification. Claim 17 is directed only to those polypeptides which are purifiable from such a medium using Flt4 affinity purification. The specification contains a working example of such an affinity purification. (See Example 5, pp. 17-19.)

The remaining rejected claims all depend from amended claims 1, 14, or 17. Thus, the amendments to claims 1, 14, and 17 are sufficient to overcome the Patent Office's rejections of all of claims 1, 13-15, and 17-19, and the rejections should be withdrawn.

**XIII. The rejection under §102(a) based upon Pajusola *et al.* was improper, because the cited reference fails to disclose a purified polypeptide capable of binding with high affinity to Flt4.**

In paragraph 19 of the Office action, the Patent Office rejected claim 1 under §102(a), asserting that the claim is anticipated by Pajusola *et al.*:

Claim 1 is rejected under 37 U.S.C. § 102(a) as being anticipated by Pajusola *et al.*

Pajusola *et al.* teach at p. 3550, column 1, a purified polypeptide, colony stimulating factor-1 (CSF-1), which binds a CSF-1 receptor/Flt4 fusion protein. An inherent

XII. The rejection of claims 1, 13-15, and 17-19 under §112, first paragraph, should be withdrawn because the specification enables one to make the invention commensurate in scope with these claims.

In paragraph 16 of the Office action, the Patent Office rejected claims 1, 13-15, and 17-19 under 35 U.S.C. §112, first paragraph, asserting that the specification fails to enable one skilled in the art to make the invention commensurate in scope with these claims:

Claims 1, 13-15 and dependent claims 17-19 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for the scope of claim 16, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at p. 8.)

The Applicants traverse in part and amend in part.

A number of the Patent Office's bases for rejecting claims 1, 13-15, and 17-19 relate to the amount of experimentation required to make and test polypeptides. As explained in detail in Part XI, above, the experimentation that is required is routine screening, and the specification enables *in vitro* screening assays which employ Flt4 protein or the extracellular domain thereof. In such circumstances, the Patent Office's reviewing court tolerates a considerable amount of experimentation under §112, first paragraph. See *In re Wands, supra*. Under the guidelines established in *In re Wands*, the conclusion that *undue* experimentation is required was improper.

Moreover, the Applicants have amended claims 1, 14, and 17 herein to overcome the Patent Office's rejections. In its rejection, the Patent Office asserts, "claim 1 encompasses all proteins that may interact with the Flt4 receptor." However, claim 1 is not directed to all proteins that may interact with the Flt4 receptor, but only to "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." Thus, amended claim 1 is commensurate in scope with the specification, which teaches a high affinity ligand which binds the extracellular domain of Flt4.

encompassed fragments and test their ability to bind the receptor" ignores the fact that the required experimentation is rendered routine by the Flt4 *in vitro* screening assays of the specification.

The Patent Office's rationale that "a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor" also ignores the scientific ability of one of ordinary skill in the art. Importantly, one of ordinary skill in the art would not conduct experimentation by haphazardly making all of the possible fragments of SEQ ID NO: 33 and testing their ability to bind the receptor. An artisan of ordinary skill understands that each fragment that is screened provides guidance as to that portion of SEQ ID NO: 33 that is effective for binding, and that portion which is not.<sup>3</sup> An artisan of ordinary skill also understands techniques for accelerating a screening process,<sup>4</sup> and techniques for screening multiple polypeptides *simultaneously*. Thus, the Patent Office's reasoning greatly overstates both the quantity and the nature of the experimentation required to practice the invention as claimed.

For all of these reasons, the specification enables one of ordinary skill in the art to practice the invention of claims 8 and 9, and the rejection under §112, first paragraph, should be withdrawn.

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<sup>3</sup> For example, a determination that a polypeptide comprising residues 34-180 of SEQ ID NO: 33 is effective to permit binding to Flt4 and that a polypeptide comprising residues 181-350 is ineffective to permit binding would provide significant guidance as to that portion of SEQ ID NO: 33 to further screen for effective fragments. Thus, the assertion that it would be necessary to screen "all" fragments of SEQ ID NO: 33 to practice the claimed invention relies upon the false assumption that individual screening assays will be performed without knowledge gained from prior screenings.

<sup>4</sup> For example, it is within the skill of the art to synthesize spaced deletion mutants (e.g., residues 34-350, 34-330, 34-310, etc.) from SEQ ID NO: 33, rather than successive deletion mutants (34-350, 34-349, 34-348 . . .), to more rapidly identify effective portions for binding Flt4.

The specification provides significant guidance for determining portions of SEQ ID NO: 33 that are effective to permit Flt4 binding. For example, although SEQ ID NO: 33 contains 350 amino acids, the specification provides guidance that the region critical for receptor activation is contained within its first approximately 180 amino acid residues.

By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin, et al., Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues.

(Specification, pp. 27-28.)

Additionally, the specification outlines a protocol for defining that portion of SEQ ID NO: 33 which corresponds with the naturally-occurring Flt4 ligand. (See p. 27, lines 5-22.) Third, the specification provides guidance to (a) generate progressive deletion products of the Flt4 ligand cDNA; (b) express these modified cDNAs; and (c) assay the resulting truncated protein forms, e.g., by studying their ability to induce Flt4 autophosphorylation. These teachings serve to both provide guidance for predicting the portions of SEQ ID NO: 33 that are effective to permit Flt4 binding; and (2) reduce the amount of experimentation required to determine the minimum portion of SEQ ID NO: 33 that is critical for receptor binding.

Importantly, because the specification teaches *in vitro* screening assays (employing Flt4 or Flt4 extracellular domain), the experimentation required to practice the full scope of claims 8 and 9 is routine in nature. The fact that routine screening assays are what is required, and that such assays are taught in the specification, further supports a conclusion of enablement. See *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988) ("Enablement is not precluded by the necessity for some experimentation such as routine screening. . . . The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.") The Patent Office's rationale that "a vast amount of experimentation would be required to make all the



The Patent Office's second asserted basis for rejection is set forth

below:

Second, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. The specification also teaches at Example 11 that a fragment having amino acids 1-180 of SEQ ID NO:33 *may* bind the receptor.

(Office action at p. 10.)

The foregoing quotation is a description of a working example and of guidance provided in the specification for identifying portions of SEQ ID NO: 33 effective to permit Flt4 binding. As such, the foregoing quotation supports, rather than negates, a conclusion of enablement. *See Ex parte Forman* (cited by the Patent Office).

The Patent Office's remaining rationales in support of its rejection relate to the alleged unpredictability of identifying effective portions of SEQ ID NO: 33:

Third, the skilled artisan could not predict which of the vast number of polypeptides encompassed by the claims would bind the Flt4 receptor, because the secondary structures required for interacting with the receptor were unknown and the secondary structures of any of the fragments of the claimed polypeptide were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor solely because it's 23 kD in size. Fourth, a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor. Thus, an undue amount of experimentation would be required to make and use the claimed invention.

(Office action at p. 10.)

However, these assertions ignore guidance provided in the specification for determining which portions of SEQ ID NO: 33 are required to permit binding to Flt4, and further ignore the nature of experimentation that one skilled in the art would conduct to identify such fragments.

8, requires that the polypeptide have an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

By providing the amino acid sequence set forth in SEQ ID NO: 33, the specification enables one skilled in the art to make essentially any polypeptide comprising a portion of SEQ ID NO: 33. For example, such polypeptides may be synthesized using automated peptide synthesizers or using recombinant techniques (e.g., using polynucleotides of the invention). Moreover, the specification enables binding assays to determine whether a polypeptide that has been synthesized is capable of binding to Flt4 receptor tyrosine kinase, and enables phosphorylation assays to determine whether such a polypeptide is capable of stimulating Flt4 autophosphorylation (see, e.g., Example 4). For these reasons alone, the specification enables one to make the invention of claims 8 and 9, and the rejection under § 112, first paragraph, must be withdrawn.

Moreover, the Patent Office's reasoning does not support the present rejection of the amended claims. The Patent Office's first basis for rejection is that "claim 8 encompasses fragments of all sizes from all locations of the protein of SEQ ID NO:33, and claim 9 encompasses all of these same fragments having an apparent molecular weight of 23 kD." However, claim 8 does not encompass fragments of all sizes and locations of the protein of SEQ ID NO: 33. Rather, claim 8 encompasses only polypeptides which comprise *a portion of SEQ ID NO: 33 effective to permit binding to an Flt4 receptor tyrosine kinase*. As the Patent Office acknowledges in its rejection, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor. Thus, the scope of amended claim 8 (and claim 9 which depends therefrom) is narrower than the Patent Office has asserted.<sup>2</sup>

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<sup>2</sup> By teaching that polypeptides of the invention are Flt4 ligands, and by teaching how to make and use Flt4 protein (and/or the extracellular fragment thereof), the specification enables one skilled in the art to perform the routine screening necessary to identify those portions of SEQ ID NO: 33 that are effective to permit Flt4 binding.

fragments of claims 9 and 10, for example, appear to encompass only part of SEQ ID NO:33.

(Office action at p. 8.)

The Applicants respectfully submit that the definition of the term "fragment" asserted by the Patent Office is contrary to the understanding of the artisan of ordinary skill. However, claim 10 has been canceled without prejudice and claims 8 and 9 have been amended such that neither claim recites "fragment." Accordingly, the rejection for indefiniteness has been rendered moot.

D. Summary

All of the rejections under §112, second paragraph have been rendered moot by the amendments herein. Accordingly, these rejections should be withdrawn.

- XI. The rejection of claims 8 and 9 under §112, first paragraph, should be withdrawn because the specification enables one to make the invention commensurate in scope with these claims.

In paragraph 17 of the Office action, the Patent Office rejected claims 8 and 9 under 35 U.S.C. §112, first paragraph, asserting that the specification fails to enable one skilled in the art to make the invention commensurate in scope with these claims:

Claims 8 and 9 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for the scope of claim 10, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with it is most nearly connected, to make the invention commensurate in scope with these claims.

(Office action at p. 10.)

The Applicants respectfully traverse.

Amended claim 8 recites, "A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Claim 9, which depends from claim

demonstrates that the indefiniteness objection to the phrase "specifically binds" is without merit.

Notwithstanding the foregoing, the Applicants have adopted the Patent Office's suggested language and have amended claim 1 herein to recite, "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." The adoption of the "high affinity" language -- which was suggested by the Patent Office and which "the art can define" -- renders the rejection of claim 1 moot.

The amendments set forth herein also render the objection moot with respect to claim 8. Claim 8 has been amended to recite, "A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding." Thus, claim 8 requires binding to Flt4 and requires that the polypeptide comprise a portion of SEQ ID NO: 33 effective to permit such binding. Because claim 8 no longer recites "specifically binds," the rejection based upon indefiniteness is rendered moot.

B. The rejection of claim 10 has been rendered moot.

In paragraph 14 of the Official action, the Patent Office rejected claim 10, asserting that the term "approximately" in claim 10 rendered the claim indefinite. (Office action at p. 7.) Claim 10 has been canceled herein without prejudice, rendering this rejection moot.

C. The rejection of claims 8-10 relating to the term "fragments" has been rendered moot.

In paragraph 15 of the Office action, the Patent Office rejected claims 8-10, asserting that the claims were indefinite:

Claims 8-10 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, fragments of a polypeptide comprising SEQ ID NO:33 are claimed. As such, the fragments must consist of *at least* the amino acids of SEQ ID NO:33. It is unclear whether this is the intended meaning, because the

does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

The art can define high affinity and non-specific binding to receptors; however, the instant inventions encompasses numerous polypeptides which would be expected to have affinities for the receptor anywhere between high affinity to non-specific binding. Intermediate affinities are not definable without a standard of affinity for "specific" binding, and hence it is impossible to determine whether such compounds would be included within the bounds of the claims.

(Office action at pp. 6-7.)

Contrary to the Patent Office's assertions, one skilled in the art understands from the specification that "specific" binding is an indication of high affinity binding. For example, Example 5 teaches that an approximately 23 kD polypeptide is isolatable from PC-3 conditioned medium via affinity chromatography, using the Flt4 extracellular domain in the affinity matrix. This polypeptide was found only in chromatographic fractions associated with Flt4 stimulating activity. All other components in the chromatographic fractions containing stimulatory activity were also distributed in the starting material and in small amounts in other washing and elution steps. "Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4." (Specification at p. 19, lines 6-8.) Thus, the specification provides an indication that the ability to affinity purify is a measure of specific binding. The specification also provides the guidance that the isoforms of VEGF "do not show specific binding to Flt4." (Specification at p. 4, lines 24-25.)

The Patent Office's own rejection is evidence that "specific" binding is understood to be an indication of high affinity binding. For example, the Patent Office asserts, "The art can define high affinity and non-specific binding to receptors." (Office action at p. 7.) Here, the Patent Office acknowledges that the art can define "high affinity binding" to receptors and can define its antithesis, "non-specific binding to receptors." Implicit in the Patent Office's own reasoning is an understanding that "specific" binding is high affinity binding, which the art can define. Thus, the Office action itself

The Applicants respectfully traverse.

As recognized by the Patent Office, "The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation." Thus, the Patent Office acknowledges that the specification contains a working example of a polypeptide meeting the "capable of stimulating Flt4 phosphorylation" limitation of claim 14.

Additionally, the specification teaches *in vitro* assays useful for screening polypeptides for their ability to stimulate Flt4 phosphorylation. (See, e.g., Example 4 (pp. 15-17) of the specification. The teaching of an Flt4 phosphorylation assay serves to precisely define the metes and bounds of claim 14, making the functional language perfectly acceptable. See M.P.E.P. §2173.05(g). As outlined in the remarks below (see Parts XI and XII) concerning the issue of enabling disclosure, the specification satisfies the enabling disclosure requirements of the patent statute by virtue of its teachings relating to Flt4 ligands, coupled with its teachings relating to screening assays involving Flt4 protein (or the extracellular domain thereof). Accordingly, the Applicants respectfully request withdrawal of the Patent Office's remarks relating to non-enablement.

X. The rejections of claims 1 and 8-10 under 35 U.S.C. §112, second paragraph, as being indefinite should be withdrawn.

In paragraphs 13-15 of the Office action, the Patent Office rejected claims 1 and 8-10, asserting that these claims were indefinite. Claims 9-10 and 13-19 also were rejected because of their dependence from claims 1 and/or 8. As set forth below, the foregoing amendments to the claims render all of these rejections moot.

A. The rejection of claims 1 and 8 should be withdrawn.

In paragraph 13, the Patent Office rejected claims 1 and 8, asserting that the term "specifically" therein rendered the claims indefinite:

The term "specifically" in claims 1 and 8 is a relative term which renders the claim indefinite. The term "specifically" is not defined by the claim, the specification

Claim 17 recites, "A purified and isolated polypeptide according to claim 1, *said polypeptide being purifyable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase* . Thus, whereas claim 1 is directed to any purified and isolated polypeptide that is capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase, claim 17 further requires that the polypeptide be purifyable from a conditioned media from a particular cell line. The Applicants respectfully submit that not every polypeptide that satisfies the requirements of claim 1 is also purifyable from conditioned media of the particular cell line recited in claim 17. Thus, the limitation as to source carries with it inherent structural limitations: the structure of the Flt4 ligand polypeptides that are isolatable from PC-3 medium. Since claim 17 further limits claim 1, the objection thereto should be withdrawn.

IX. The objection to claim 14 has been rendered moot.

In paragraph 12 of the Office action, the Examiner objected to claim 14 under 37 C.F.R. §1.175(c), asserting that the claim failed to limit the subject matter of claim 13. (Office action at p. 6.) Claim 14 has been amended herein to read as an independent claim. Accordingly, the Patent Office's objection is rendered moot, and should be withdrawn.

In its objection, the Patent Office also made assertions concerning the issue of enabling disclosure:

Claim 14 recites the functional limitation of stimulating Flt4 phosphorylation, which is a functional limitation not enabled by the teachings of the specification (M.P.E.P. §2173.05(g)). The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation at Figure 5. The specification does not teach necessary or sufficient structures of the 23 kD protein that promote phosphorylation, nor could the skilled artisan have predicted what these structures would be from the state of the art at the time of the invention.

(Office action at p. 6.)

**VII. The objection to the terminology at page 27, line 24, should be withdrawn.**

In paragraph 9 of the Office action, the Examiner asserted that less than preferable terminology was employed at page 27, line 24, of the specification:

The disclosure is objected to because of the following informalities: 3' deletions of the Flt4 ligand are taught at p. 27, line 24, but deletions occurring at the end of a protein are preferably termed "C-Terminal" or "carboxy terminal" deletions. "3'" is preferred for describing nucleic acids only. Appropriate correction is required.

(Office action at p. 5.)

The applicants respectfully submit that page 27, lines 24-26, refer to "progressive 3' deletions in the 3' coding sequences of the Flt4 ligand precursor clone, resulting in COOH-terminal truncations of its protein product." Thus, the specification uses the phrase "3' deletions" to describe alteration of a nucleic acid encoding a protein, and use the phrase "COOH-terminal truncations" for describing deletions at the end of the protein encoded thereby. In the present amendment, the Applicants have deleted "COOH-terminal" and substituted therefor the "carboxy-terminal" language preferred by the Patent Office. Accordingly, the specification is in conformity with the preferred language noted by the Patent Office, and the objection thereto should be withdrawn.

**VIII. The objection to claim 17 should be withdrawn because claim 17 provides further limitation to claim 1 from which it depends.**

In paragraph 11 of the outstanding Office action, the Patent Office objected to claim 17, asserting that claim 17 failed to further limit claim 1 from which claim 17 depends:

Claim 17 is objected to under 37 C.F.R. § 1.75(c) for not further limiting the subject matter of claim 1.

Specifically, the instant claim does not recite further structural limitations to the polypeptide of claim 1; the particular source of the polypeptide is *de minimus* [sic].

(Office action at p. 6.)

The Applicants respectfully traverse.



kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase. As explained in part A, above, the '011 application teaches how to make and use such a polypeptide. Accordingly, claim 14 is afforded priority by the '011 application. Claim 23, which depends from claim 14 and contains a molecular weight limitation ("approximately 23 kD"), is afforded priority to the '011 application for the reasons described above for claim 13.

As the foregoing discussion of the '011 preliminary amendment indicates, several claims in the present application, including at least claims 1, 13, 14, 16, 17, and 19, are entitled to priority from the '011 application. The Applicants respectfully request appropriate correction of the Patent Office's priority determination in the next action on the merits in the present application.

**V. A copy of the Inventors' supplemental declaration has been filed herewith.**

In paragraph 7 of the Office action, the Examiner indicated that the supplemental inventor's declaration filed with the Preliminary Amendment dated August 12, 1996, was misplaced or omitted during transmittal. A copy of the supplemental inventor's declaration is filed herewith to complete the Patent Office's file.

**VI. The Applicants will file formal drawings upon receipt of a notice of allowability.**

In paragraph 8 of the Office action the Patent Office suggests that, "Pursuant to a change in Office policy effective 25 April 1996, *formal drawings will be required at the time allowable subject matter is first indicated.*" Notwithstanding the foregoing, and the fact that claims 2 and 12 in the application have been allowed, the Applicants submit that formal drawings are not required until issuance of a Notice of Allowability, i.e., until the application is allowed. See 37 C.F.R. §1.85 and M.P.E.P. §608.02(b). In a telephone interview between the Examiner and the undersigned attorney, which the Applicants acknowledge with thanks, the Examiner confirmed that formal drawings were not required in response to the outstanding Office action.

35 U.S.C. §112. . . . [T]he later explicit description of an inherent property does not deprive the product of the benefit of the filing date of the earlier application."); see also *Application of Davies*, 177 U.S.P.Q. 381, 385, 475 F.2d 667, 671-72 (C.C.P.A. 1973) ("[W]e see no impediment to the present applicants' refiling their application and incorporating a discussion of the allegedly unobvious properties [of the invention] while retaining the effective date of the application involved here through §120.")

Claim 16 depends from claim 13 and recites that "amino terminal amino acids 2 through 18 of said polypeptide have an amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ ID NO: 13." The recited amino acid sequence is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from PC-3 conditioned medium.<sup>1</sup> As such, the inclusion of this property in the present application and in claim 16 does not deprive claim 16 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, and *Application of Davies, supra*.

Claim 17 depends from claim 1 and requires that the polypeptide be purifiable from PC-3 conditioned media (PC-3 cell line ATCC CRL No. 1435) using an affinity purification procedure wherein the affinity purification matrix comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase. As explained in detail in subpart A, above, the '011 application teaches in detail the procedure recited in claim 17 for purification of such a polypeptide. Accordingly, claim 17 is afforded priority by the '011 application.

Claim 19 depends from claim 1 and requires a detectable label. Detectably labeled Flt4 ligands were specifically contemplated as an aspect of the '011 application. (See e.g., Preliminary Amendment to '011 application at pp. 18-19 (claims 33-35).) Accordingly, claim 19 is afforded priority by the '011 application.

Independent claim 14 of the present application recites, "A purified and isolated polypeptide which is capable of binding to Flt4 receptor tyrosine

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<sup>1</sup> Moreover, the '011 application teaches that the amino terminal amino acid sequence should be determined. (See Preliminary Amendment to '011 application at p. 15 (Example 15).)

for screening the cDNA library using, e.g., oligonucleotide probes generated based upon the peptide sequences of purified Flt4 ligand.

Significantly, the '011 application also contained claims to an Flt4 ligand and to methods of using an Flt4 ligand. (See preliminary amendment to '011 application at pp. 18-19.) Uses for an Flt4 ligand that are taught in the '011 application include use for the detection of Flt4 (increased Flt4 expression being observable in metastatic lymph nodes and lymphangiomas); use for regulating the growth and functions of certain endothelial cells, especially lymphatic endothelia; and use for assaying for inhibitors. (See, e.g., Preliminary Amendment to '011 application at pp. 6-7 and 19.) Thus, when one considers the entirety of the '011 application, including its preliminary amendment, one finds abundant §112, first paragraph, support for a purified Flt4 ligand as claimed in the present application.

- B. Several claims in the present application are properly afforded priority to the '011 application.

Referring to the present application, claim 1 is directed to "A purified and isolated polypeptide capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase." As explained in detail in subpart A, above, the '011 application teaches how to make and to use such a polypeptide. Accordingly, claim 1 is afforded priority by the '011 application.

Claim 13 depends from claim 1 and contains the limitation that the polypeptide have "an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions." The approximate 23 kD molecular weight is an *inherent property* of the Flt4 ligand that the '011 application teaches one how to purify from the PC-3 conditioned medium. As such, the inclusion of this property in the present application and in claim 13 does not deprive claim 13 of being afforded priority to the '011 application. See *Therma-Tru Corp. v. Peachtree Doors Inc.*, 33 U.S.P.Q.2d 1274, 1276, 44 F.3d 988 (Fed. Cir. 1995) ("A claim in a CIP application is entitled to the filing date of the parent application when the claimed invention is described in the parent specification in a manner that satisfies, inter alia, the description requirement of

activated Sepharose) is capable of removing the component responsible for stimulating Flt4. (Preliminary amendment to '011 application at p. 11.) Based on the experimental results reported in Example 12, the '011 application states, "These data prove that PC-3 cells produce soluble ligand for FLT4. The above experiments prove that the ligand binds to the recombinant FLT4 EC domain. Thus, that ligand can be purified using the recombinant FLT4 EC domain in affinity chromatography. The purified protein can be electrophoresed in SDS-PAGE, blotted onto polyvinylidene difluoride (PVDF) membranes and its amino terminal sequence can be determined by methods standard in the art." (Preliminary amendment to '011 application at p. 11.) Thereafter, Example 12 contains teachings as to the determination of peptide sequences of the purified ligand and identification and cloning of a cDNA encoding the ligand.

Example 15 in the '011 application is directed to "Purification and sequencing of the Flt4 ligand." (Preliminary amendment to '011 application at p. 15.) Example 15 teaches that the PC-3 conditioned medium is concentrated and loaded onto a column of immobilized Flt4 extracellular domain. One embodiment taught in Example 15 is an affinity matrix comprising the Flt4 extracellular domain cross-linked to CNBr-activated Sepharose, i.e., the same affinity matrix employed in Example 5 in the present application. Example 15 in the '011 application teaches that chromatographic fractions are tested for the ability to stimulate tyrosine phosphorylation of Flt4, as was done in Example 5 of the present application. Thus, Example 15 in the '011 priority document teaches an affinity purification procedure for purifying a Flt4 ligand of the invention.

Example 15 in the '011 application further directs, "The purified biologically active ligand protein is microsequenced and the degenerate oligonucleotides are made based on the amino acid sequence obtained." (Preliminary amendment to '011 application at p. 15.) Example 16 of the '011 application is directed to constructing a cDNA library from PC-3 cells, to be screened for a cDNA encoding the Flt4 ligand. (Compare Example 6 in the present application.) Example 17 in the '011 application provides procedures

withdrawn claims be canceled, as part of a "complete response to the final rejection," is premature.

- IV. Several claims in the application are entitled to priority based on U.S. Patent Application Serial No. 08/340,011, when one considers the entirety of the '011 application, including the preliminary amendment thereto.

In paragraph 6 of the Office action the Patent Office asserted that "no claims are afforded priority to application Serial No. 08/340011" (hereinafter "the '011 application"). (Office action at p. 4.) The Applicants respectfully traverse, because several claims in the application are entitled to priority based on the '011 application, when one considers the entirety of the '011 application, including the preliminary amendment thereto.

- A. The '011 priority application contains numerous teachings related to an Flt4 ligand that the Patent Office failed to consider in its priority determination.

The '011 application was a Rule 62 continuation-in-part of an earlier application, and was filed with a preliminary amendment containing a disclosure of an Flt4 ligand. (See preliminary amendment to the '011 application dated November 14, 1994.) The Patent Office's stated rationale for its determination that the '011 application affords no priority to the present application cites only pages 7-8 of the '011 application, suggesting that the more pertinent preliminary amendment was never considered.

Examples 12-17 in the '011 application all were introduced in the preliminary amendment portion thereof, and all are highly pertinent to the claim of priority in the present application. For example, Example 12 in the '011 application teaches that a conditioned media from the PC-3 prostatic adenocarcinoma cell line (ATCC CRL 1435) is capable stimulating Flt4 autophosphorylation in NIH3t3 cells expressing Flt4. Example 12 further teaches that a "flow-through fraction" of concentrated conditioned medium (i.e., the fraction containing proteins of less than 10,000 molecular weight) was not responsible for stimulating the Flt4 phosphorylation, and that pretreatment of the conditioned medium with Flt4 extracellular domain (coupled to CNBr-

reserve the right to claim such subject matter in other applications, such as continuations, continuations-in-part, and divisional applications.

II. The Patent Office's basis for maintaining the restriction requirement is inconsistent with the patent statutes.

In paragraphs 1 and 2 of the Office action, the Patent Office maintained the Restriction Requirement first imposed in the Office Action mailed May 29, 1996, citing as support various provisions of the M.P.E.P., including §802.01. The Applicants respectfully submit that, to the extent that the statutory provisions of 35 U.S.C. §121 ("independent and distinct") are inconsistent with the M.P.E.P. provisions cited by the Examiner ("may properly be divided if . . . 'distinct' inventions, even though dependent"), it is the statutory provisions which are controlling, because statutory provisions have the force of law. "The Manual does not have the force of law or the force of the Patent Rules of Practice in Title 37 Code of Federal Regulations." (See Forward to M.P.E.P., 6th Ed., Rev. 2, July, 1996.) Because the asserted basis for maintaining the restriction requirement is contrary to the plain language of the governing patent statute, reconsideration and withdrawal of the restriction requirement is respectfully requested.

III. The requirement that non-elected claims be canceled is premature.

In paragraph 3 of the Office action, the Patent Office required cancellation of non-elected claims or other appropriate action:

This application contains claims 3, 4, 6 (each amended), 5, 7, and 11 drawn to inventions non-elected with traverse in Paper No. 9. A complete response to the final rejection must include cancellation of non-elected claims or other appropriate action (37 C.F.R. §1.144; M.P.E.P. §821.01).

(Office action at p. 3.)

The Applicants respectfully submit that the outstanding Office action is the first Office action on the merits and is not a "final rejection." Only the restriction requirement has been made final. Accordingly, the requirement that the

## REMARKS

### I. History of claims and explanation of amendments.

The application as filed contained twelve claims. In a preliminary amendment (Paper No. 10) dated August 12, 1996, claims 1-4, 6, 8-9, and 12 were amended, and claims 13-19 were added to the application. In the present amendment, the Applicants cancel claims 10 and 18; amend claims 1, 8, 9, 14, and 17; and add new claims 20-25. Thus, claims 1-9, 11-17, and 19-25 are pending. Claims 3-7 and 11 have been withdrawn from consideration as being drawn to a non-elected invention.

All of the amendments herein find support in the application as originally filed. Most of the amendments to the specification correct obvious typographical and grammatical errors and the like, as requested by the Patent Office in paragraph 10 of the Office action. Support for the amendment at page 13, line 22, is found in the specification at page 13, line 18. Support for the amendment at page 19, line 2, is found in the specification at page 18, lines 16-24, for example.

Amended Figure 10 differs from Figure 10 as originally filed only in that two "N" residues have been underlined in amended Figure 10. The two underlined "N" residues conform to art-recognized N-linked glycosylation sequences (i.e., Asn-X-Ser or Asn-X-Thr). Support for this amendment is found at page 26, lines 21-24 in the specification. A separate letter requesting amendment of the drawing has been filed herewith.

The amendments to claim 1 find support in Example 5 (e.g., at p. 17, lines 13-15); at p. 4, line 6; and elsewhere throughout the specification.

The amendments to claim 8 and 14, which formerly depended from claims 2 and 1, find support in claims 2 and 1 as originally filed, as well as elsewhere throughout the specification.

The amendments to claim 17 find support in Example 5 (e.g., at p. 17, lines 13-15) and elsewhere throughout the specification.

The Applicants do not intend by these or any other amendments to abandon the subject matter of any claim as originally filed or later amended, and

comprises a polypeptide comprising the extracellular domain of Flt4 receptor tyrosine kinase.

-- 20. A polypeptide according to claim 8 which is capable of binding the extracellular domain of Flt4 receptor tyrosine kinase with high affinity and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

21. A polypeptide according to claim 8 further comprising a detectable label.

22. A pharmaceutical composition comprising a polypeptide according to claim 8 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.

23. A polypeptide according to claim 14 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

24. A polypeptide according to claim 14 comprising a portion of SEQ ID NO: 33 effective to permit binding to Flt4 receptor tyrosine kinase and stimulation of Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

25. A pharmaceutical composition comprising a polypeptide according to claim 14 in a pharmaceutically-acceptable diluent, adjuvant, or carrier. --



In the drawing:

Please delete Figure 10 and substitute therefor amended Figure 10 filed herewith.

In the claims:

Please cancel claims 10 and 18; amend claims 1, 8, 9, 14, and 17; and add new claims 20-25 as shown below:

1. (Twice amended) A purified and isolated polypeptide [which specifically binds] capable of binding with high affinity to the extracellular domain of Flt4 receptor tyrosine kinase.

8. (Twice amended) A polypeptide capable of binding to an Flt4 receptor tyrosine kinase, said polypeptide comprising a portion of SEQ ID NO: 33 effective to permit such binding (fragment of the purified and isolated polypeptide according to claim 2 which is capable of specifically binding to an Flt4 receptor tyrosine kinase).

9. (Twice amended) (The fragment) A polypeptide according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

14. (Amended) A purified and isolated polypeptide [according to claim 13] which is capable of binding to Flt4 receptor tyrosine kinase and stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor tyrosine kinase.

17. (Amended) A purified and isolated polypeptide according to claim 1, said polypeptide being purifiable from conditioned media from a PC-3 prostatic adenocarcinoma cell line, said cell line having ATCC CRL No. 1435, using an affinity purification procedure wherein the affinity purification matrix

At page 24, line 12, please delete "1xSSC" and substitute therefor -- 1x SSC --.

At page 25, line 22, please delete "used". In the same line, please delete "and to" and substitute therefor -- and used to --.

At page 26, line 2, please delete "ul" and substitute therefor --  $\mu$ l --

At page 26, line 24, please delete "marked" and substitute therefor -- underlined --.

At page 26, line 28, please delete "BRF3" and substitute therefor -- BRP3 --.

At page 27, line 25, please delete "COOH-terminal" and substitute therefor -- carboxy-terminal --.

At page 27, line 28, please delete "asd" and substitute therefor -- as --.

At page 28, line 29, please delete "42C" and substitute therefor -- 42°C --.

At page 29, line 3, please delete "52C" and substitute therefor -- 52°C --.

At page 28, line 4, please delete "70C" and substitute therefor -- 70°C --.

## AMENDMENTS

### In the specification:

At page 3, line 30, after "ingrowth stage" please insert a period -- .

At page 6, line 19, please delete "election" and substitute therefor -- electron --.

At pages 12, lines 9, 10, and 19, please delete "ug" and in each instance substitute therefor --  $\mu$ g --.

At page 13, line 22, please delete "S68203" and substitute therefor -- X68203 --.

At page 17, line 14, please delete "recombinant-produced" and substitute therefor -- recombinantly-produced --.

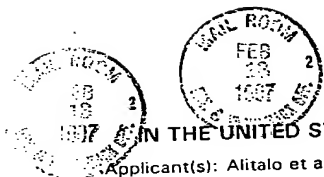
At page 19, line 2, after "lanes 8 and 9" please insert -- in Figure 6

At page 19, line 27, please delete "ug" (both instances), and in each instance substitute therefor --  $\mu$ g --.

At page 20, line 11, and page 21, line 17, please delete "ug" and in each instance substitute therefor --  $\mu$ g --.

At page 21, line 16, please delete "design" and substitute therefor -- designed --.

At page 24, line 11, please delete "5xSSPE" and substitute therefor -- 5x SSPE --.



PATENT  
28967/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/510,133

Filed: August 1, 1995

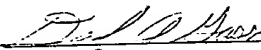
Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

) I hereby certify that this paper is  
) being deposited with the United  
) States Postal Service with sufficient  
) postage as first class mail in an  
) envelope addressed to: Assistant  
) Commissioner for Patents,  
) Washington, D.C., 20231 on this  
) date:

) Date: February 10, 1997

)   
) David A. Gass  
) Registration No. 38,153  
) Attorney for Applicants

AMENDMENT AND REPLY PURSUANT TO 37 C.F.R. §§ 1.111 AND 1.115

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

In an Office action mailed September 10, 1996, the Patent Office allowed claims 2 and 12, but rejected claims 1, 8-10, and 13-19 variously under 35 U.S.C. §§ 102(a), 102(b), and 112, first and second paragraphs. The Applicants respectfully request reconsideration in light of the following amendments and remarks.

U.S. PATENT OFFICE  
WASHINGTON, D.C. 20503

3. **Fee for Claims**

The fee for additional claims [(37 CFR 1.16(b)-(d))] has been calculated as shown below:

					SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	Claims Remaining After Amendment	Highest No. Previously Paid For		Present Extra	Rate	Additional Fee	Rate	Additional Fee
TOTAL	23	MINUS	20	= 3	X11 =		X22 =	\$ 66.00
INDEP.	5	MINUS	3	= 2	X40 =		X80 =	\$160.00
11	Total Presentation of Multiple Dependent Claim				+ 130 =		+ 260 =	
TOTAL ADDITIONAL FEE							OR	\$226.00

4. **Method of Payment of Fees**

- ☒ Attached is a check in the amount of: \$226.00
- ☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

5. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By: \_\_\_\_\_

David A. Gass  
Reg. No. 38,153

February 10, 1997

1. Small Entity Status

- ☐ Verified statement(s) claiming small entity status is(are) attached.
- ☐ Small entity status has been established and is still effective.
- ☒ Has not been established.

2. Extension of Time

- ☒ This is a petition for an extension of time under 37 CFR 1.136 for the total number of months checked below:

EXTENSION (Months)		FEE FOR LARGE ENTITY		FEE FOR SMALL ENTITY
One Month		\$110.00		\$55.00
Two Months	x	\$390.00		\$195.00
Three Months		\$930.00		\$465.00
Four Months		\$1,470.00		\$735.00

If an additional Extension of Time is required, please consider this a petition therefor.

Extension Fee: \$390.00

- ☐ An extension for \_\_\_\_\_ month(s) has already been secured and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested.

Deduction: \$

Extension Fee Due With This Request \$



**PATENT**  
**ATTORNEY DOCKET NO. 28967/32863**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): ) Title: RECEPTOR LIGAND  
Alitalo et al. )  
Serial No: 08/510,133 ) Group Art Unit: 1814  
Filed: August 1, 1995 ) Examiner: Lathrop, B.

**RECEIVED**  
JTB 27 1997  
Cheney 10/27

**AMENDMENT TRANSMITTAL WITH  
PETITION FOR EXTENSION OF TIME**

*Assistant Commissioner for Patents  
Washington, D.C. 20231*

Sir:

Transmitted herewith for filing in the above-identified application are the following:

1. Amendment and Reply Pursuant to 37 C.F.R. §§1.111 and 1.115;
2. Information Disclosure Statement, including Form PTO-1449 and copies of documents B1-B5 and C14-C110;
3. Check for \$390.00 in payment of fee for Two Months Extension of Time;
4. Check for \$226.00 in payment of fee for extra claims;
5. Check for \$230.00 in payment of fee or consideration of IDS;
6. Copy of Inventors' Declaration; and
7. Request for Amendment of Drawing, including amended Figure 10 and copy of Figure 10 as filed, marked to show amendments.

**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on February 10, 1997, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

David A. Gass

1 50

PDGF-A .MRTWACLLL LGGCYLAHAL AEEAETPREL IERLARSQIH SIRDLOQLLE  
 PDGF-B MNRCWA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSHHSIR SFDDLQRLH  
 PLGF .....MP VMRLFPCTLO LLAGLAL...  
 VEGF ..... MNFLLSWVH WSLALLLYLH  
 FLT4-L ..... MTVLYPEYWK MYCQLRKGG

51 100

PDGF-A IDSUGAEDAL ETSLEHGHSH AINHVPEKRP VPIRRKRSI. ....EEAIP  
 PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELES... .LARGRRSLG SLTIAEPAMI  
 PLGF PAVPPQOWAL SA..... GNGSSEVEVV P.FQEVWG... ..R  
 VEGF HAKWSQAAPH AE..... GGGQNHHEVV K.FMDVYQ... ..K  
 FLT4-L WQHNRQANL NSRTEETIKF AAAHYNTEIL KSIDNEWR... ..K

101 150

PDGF-A AVKTRTVIY EIPRSQVDPT SANFLIMHFC VEVKRCIGCC NTSSVKCOPS  
 PDGF-B AEKTRTEVF EISRLIDRT NANFLVWHP VEVORCSGCC NNRNVQCRPT  
 PLGF SYRALERLV DVVSEY..PS EVEHMFSPS VSLLRCHGCC GDENLHCVPT  
 VEGF SYHPHETLV DIFQEY..PD EIEYIFKPS VPLMRCHGCC NDEGLECVPT  
 FLT4-L TCMPREVCI DVGKEF..GV ATNTFFKRC VSVYRCGCC NSEGLCHMT

151 200

PDGF-A RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE RLESG.... AT.....  
 PDGF-B QVQLRFQVVR KIEIVRKKPI FKKATVTLED HLA... ETVAAARPVT  
 PLGF ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRLEPRPLR EKMKPERC..  
 VEGF EESNITMQIM RIKPH..QGO .HIGEMSFLQ HNKLEPRPK DRARQENP..  
 FLT4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSRCMSKL DVYRQVHSII

201 250

PDGF-A .SNLNPDRH EETDVR... ..  
 PDGF-B RSPGGSQEQR AKTPQTRVTI RTVRVRPPK GKHRKFKHTH DKTALKETLG  
 PLGF ..... GDAVPRR... ..  
 VEGF ..... CGPCSERKX LFVQDPQCK CSCKNTDSRC KARQLELNER  
 FLT4-L RRSLEPATLPQ CQAANKTCPT NYMWNHICR CLAQEDFMFS SDAGDDSTDG

251 300

PDGF-A .....  
 PDGF-B A.....  
 PLGF .....  
 VEGF TCRCDKPRR... ..  
 FLT4-L FHDICGPNKE LDEETQCVC RAGLRPASCG PHKELDRNSC QCVCKNLFP

301 350

PDGF-A .....  
 PDGF-B .....  
 PLGF .....  
 VEGF .....  
 FLT4-L SQCGANREFD ENTQCVCCKR TCPRNQPLNP GKACACECTES PQKCLLRGKK

351 395

PDGF-A .....  
 PDGF-B .....  
 PLGF .....  
 VEGF .....  
 FLT4-L FHHQTCSCYR RPECTNRQAC EPGFSYSEEV CRCVPSYWKR PQMS

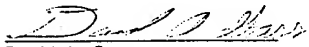
FIGURE 10



Asn-X-Ser or Asn-X-Thr). Support for this amendment is found at page 26, lines 21-24, in the specification. Entry of this amendment is requested to improve conformity between the drawing and the specification as filed.

Respectfully submitted,

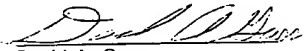
MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

  
David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

Chicago, Illinois  
February 10, 1997

11. 16.  
H. S. Gass  
UP 2-11-97  
**PATENT**  
**28967/32863**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicant(s): Alitalo et al.	)	I hereby certify that this paper is
	)	being deposited with the United
Serial No: 08/510,133	)	States Postal Service with sufficient
	)	postage as first class mail in an
Filed: August 1, 1995	)	envelope addressed to: Assistant
	)	Commissioner for Patents,
Title: RECEPTOR LIGAND	)	Washington, D.C., 20231 on this
	)	date:
Group Art Unit: 1814	)	
	)	Date: February 10, 1997
Examiner: Lathrop, B.	)	
	)	
	)	
	)	
	)	David A. Gass
	)	Registration No. 38,153
	)	Attorney for Applicants

**REQUEST FOR AMENDMENT OF DRAWING**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Attn: Official Draftsman

Dear Sir:

The Applicants respectfully request entry of an amendment to Figure 10 of the above-identified application. Three copies of Amended Figure 10 are attached hereto. Also attached is a copy of Figure 10 as filed, marked in red ink to show the amendments thereto.

Amended Figure 10 differs from Figure 10 as originally filed only in that two "N" residues have been underlined in amended Figure 10. The two underlined "N" residues conform to art-recognized glycosylation sequences (i.e.,



Second Joint Inventor, if any Vladimir Joukov	Citizenship Russia
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki	City (Zip) Same
State or Country FINLAND	State or Country Same
Date [ ] Aug 6, 1996	Signature [ ] V. Joukov

## APPLICABLE RULES AND STATUTE

### 37 CFR 1.56. DUTY OF DISCLOSURE - INFORMATION MATERIAL TO PATENTABILITY (Applicable Portion)

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentability defines, to make sure that any material information contained therein is disclosed to the Office.

Information relating to the following factual situations enumerated in 35 USC 102 and 103 may be considered material under 37 CFR 1.56(a).

### 35 U.S.C. 102. CONDITIONS FOR PATENTABILITY: NOVELTY AND LOSS OF RIGHT TO PATENT

A person shall be entitled to a patent unless --

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraph (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

### 35 U.S.C. 103. CONDITIONS FOR PATENTABILITY: NON-OBVIOUS SUBJECT MATTER

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negative by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

### 35 U.S.C. 112. SPECIFICATION (Applicable Portion)

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

## DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name: I believe that I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which was filed on August 1, 1995, as Application Serial No. 08/510,133. I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by an amendment attached hereto. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed  
☐ Yes ☒ No

(Application Serial Number)	(Country)	(Day/Month/Year Filed)
050624	Finland	13 February 1995

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)
-----------------------------	------------------------

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status: Patented, Pending or Abandoned)
08/340,011	14 November 1994	Pending

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)  
Donald J. Rest (19,490)  
Owen J. Murray (22,111)  
Allen H. Gerstein (22,218)  
Nate F. Scarpelli (22,320)  
Edward M. O'Toole (22,477)  
Michael F. Burton (25,447)

Trevor B. Juke (25,542)  
Timothy J. Vezeau (26,348)  
Carl E. Moore, Jr. (26,487)  
Richard H. Anderson (26,526)  
Patrick D. Ertel (26,877)  
James P. Zeller (28,491)  
William E. McCracken (30,195)

Richard A. Schurr (30,890)  
Anthony Nimmo (30,920)  
Christine A. Dudzik (31,245)  
Kevin D. Hogg (31,839)  
Jeffrey S. Sharp (31,879)  
Donald J. Pochopien (32,167)  
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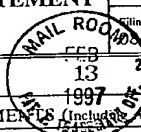
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	B5	WO 96/39515 A1	12/12/96	PCT	C12N 15/12		

**OTHER DOCUMENTS (including Author, Title, Date, Pertinent Pages, etc.)**

C14	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors and Receptors Involved in Angiogenesis," <i>The 9th International Conference of the International Society of Differentiation (ISD). Development Cell Differentiation and Cancer</i> , Pisa (Italy), September 28-October 2, 1996, p. 66 (ABSTRACT S22).
C15	Alitalo <i>et al.</i> , "Vascular Endothelial Growth Factors B and C Receptors Involved in Angiogenesis," <i>German-American Academic Council Foundation (GAAC)/Stiftung Deutsch-Amerikanisches Akademisches Konzil (DAAK), 2nd Symposium on Current Problems in Molecular Medicine: The Role of Cytokines in Human Disease</i> , November 17-20, 1996, Ringberg Castle, Germany, p. 1 (ABSTRACT).

EXAMINER R. W. 5/11/97	DATE CONSIDERED 5/11/97
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	





TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

2 of 2

FORM PTO-892 (REV. 2-92)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. <b>08/510 133</b>		GROUP/ART UNIT <b>1814</b>		ATTACHMENT TO PAPER NUMBER <b>11</b>	
NOTICE OF REFERENCES CITED				<div style="display: flex; justify-content: space-between; align-items: center;"> <span><b>2</b></span> <span><b>Alitalo et al.</b></span> </div>					
U.S. PATENT DOCUMENTS									
*		DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE		
	A								
	B								
	C								
	D								
	E								
	F								
	G								
	H								
	I								
	J								
	K								
FOREIGN PATENT DOCUMENTS									
*		DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. PP. DWG. SPEC.	
	L								
	M								
	N								
	O								
	P								
	Q								
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)									
R		<i>Rajassia et al. Signalling properties of FLT4, a proteolytically processed receptor tyrosine kinase related to two VEGF receptors. Oncogene, Vol. 9, No. 12, pages 3545-3555. 12/94</i>							
T		<i>Golikov et al. The FLT4 gene encodes a transmembrane tyrosine kinase related to the vascular endothelial growth factor receptor. Oncogene, Vol. 8, No. 5, pages 1233-1240. 5/93</i>							
EXAMINER		DATE							
		8/30/96							
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)									

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

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FORM PTO-892 (REV. 2-92)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. <b>08/510133</b>	GROUP/ART UNIT <b>1914</b>	ATTACHMENT TO PAPER NUMBER <b>11</b>		
NOTICE OF REFERENCES CITED				APPLICANT(S) <b>Alitalo et al.</b>				
U.S. PATENT DOCUMENTS								
*	DOCUMENT NO.	DATE	NAME	CLASS	SUB-CLASS	FILING DATE IF APPROPRIATE		
A	5336671	6/26/94	Ferrara et al.	435	240.1			
B	5219739	6/15/93	Tischer et al.	435	69.4			
C								
D								
E								
F								
G								
H								
I								
J								
K								
FOREIGN PATENT DOCUMENTS								
*	DOCUMENT NO.	DATE	COUNTRY	NAME	CLASS	SUB-CLASS	PERTINENT SHTS. DWG	PP. SPEC.
L	9504473	9/14/95	WO	Human Genome Sciences, Inc.	-	-		
M								
N								
O								
P								
Q								
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)								
R	Sitaras et al. Constitutive production of platelet-derived growth factor-like protein by human prostate carcinoma cell lines. Cancer Research. Vol. 48, No. 7, pp. 1730-1735. 4/1/88							
T	Fournier et al. Mutation of tyrosine residue 1337 abrogates ligand-dependent transforming capacity of the FLT4 receptor. Oncogene. Vol. 11, No. 5, pages 921-931. 9/7/95							
EXAMINER		DATE						
Brian Lathrop		8/30/96						
<p style="text-align: center;">* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)</p>								

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23. Claims 8-10 would be allowable if rewritten to overcome the rejection under 35 U.S.C. § 112 and to include all of the limitations of the base claim and any intervening claims.


*Conclusion*

24. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop whose telephone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice mail messages within 24 hours. Alternately, the examiner's supervisor, Robert A. Wax, can be reached at (703) 308-4216. The FAX number for Group 1814 is (703) 305-7401.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.  
AU 1814

  
ROBERT A. WAX  
SUPERVISORY PATENT EXAMINER  
GROUP 180

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*Allowable Subject Matter*

21. Claims 2 and 12 are allowable over the prior art of record.
22. The following is an Examiner's statement of reasons for the indication of allowable subject matter:

Tischer, et al. motivate the discovery of vascular endothelial growth factors at Background of the Invention for the advantageous result of pharmaceutical compositions for promoting wound healing. Galland et al., however, teach at p. 1238, column 2, that the physiological role of the Flt4 receptor was unknown at the time of the invention, complicating the search for a specific ligand. Fournier et al., herein cited as evidence of the state of the art at the time of the invention, further teach at p. 921, column 2, that a specific ligand for Flt4 receptor had not been identified at the time of the invention and that known vascular endothelial growth factors did not specifically bind Flt4 receptor. Human Genome Sciences, Inc. published the sequence of a Flt4 receptor ligand with greater than 99 percent identity to the instant ligand, but the date of publication does not antecede the filing date of the instant application. The ligand of Human Genome Sciences, Inc was purified using expressed sequence tags without identification as encoding a vascular endothelial growth factor; therefore, one of ordinary skill in the art at the time of the invention would not have been motivated to use these art-known sequences to arrive at the instant invention.

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*Claim Rejections - 35 USC § 102*

18. The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless --

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

19. Claim 1 is rejected under 35 U.S.C. § 102(a) as being anticipated by Pajusola et al.

Pajusola et al. teach at p. 3550, column 1, a purified polypeptide, colony stimulating factor-1 (CSF-1), which binds a CSF-1 receptor/Flt4 fusion protein. An inherent property of the fusion protein is the Flt4 receptor tyrosine kinase. Pajusola et al. thus anticipate claim 1.

20. Claim 18 is rejected under 35 U.S.C. § 102(b) as being anticipated by Sitaras et al.

Sitaras et al., the whole document, teach a conditioned medium, thus anticipating the claimed invention. Because this conditioned medium is from PC-3 prostatic adenocarcinoma cells, it has the inherent property of comprising the polypeptide of claim 1.

17. Claims 8 and 9 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the scope of claim 10, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the instantly claimed invention. First, claim 8 encompasses fragments of all sizes from all locations of the protein of SEQ ID NO:33, and claim 9 encompasses all of these same fragments having an apparent molecular weight of 23 kD. Second, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. The specification also teaches at Example 11 that a fragment having amino acids 1-180 of SEQ ID NO:33 *may* bind the receptor. Third, the skilled artisan could not predict which of the vast number of polypeptides encompassed by the claims would bind the Flt4 receptor, because the secondary structures required for interacting with the receptor were unknown and the secondary structures of any of the fragments of the claimed polypeptide were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any fragment of the protein comprising SEQ ID NO:33 would bind the receptor solely because it's 23 kD in size. Fourth, a vast amount of experimentation would be required to make all the encompassed fragments and test their ability to bind the receptor. Thus, an undue amount of experimentation would be required to make and use the claimed invention.

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SEQ ID NO:33, because the position of SEQ ID NO:13 relative to the N-terminal residue is not specified. Third, the skilled artisan would predict that some proteins which would bind the Flt4 receptor would be growth factors. It was known that many different growth factors were found in PC-3 conditioned medium as taught by Sitaras et al., for example. A vast number of additional polypeptides might interact with the receptor, even if the interaction was relatively weak. The skilled artisan could not predict which of this vast number of polypeptides, or 23 kD fragments thereof, would bind the Flt4 receptor or stimulate its tyrosine kinase activity, because the secondary structures required for interacting with the receptor were unknown and the structures of most of the proteins that may interact with the receptor were unknown. As Ferrara et al. teach at column 29, lines 29-32, even endothelial growth factors of the same size may have widely different structures and potencies. Thus, the skilled artisan would not predict that any protein characterized to no other extent than having an apparent molecular weight of 23 kD would bind the receptor. Fourth, a vast amount of experimentation would be required to make and test all the encompassed polypeptides of the instant invention. Fifth, the skilled artisan could not predict which of the approximately 34 polypeptides of claim 15 would bind the receptor, because these polypeptides would have different secondary structures determined by their different compositions, and the structures required for interacting with the receptor were unknown. For the reasons set forth above, an undue amount of experimentation would be required to make the claimed invention.



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15. Claims 8-10 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Specifically, fragments of a polypeptide comprising SEQ ID NO:33 are claimed. As such, the fragments must consist of *at least* the amino acids of SEQ ID NO:33. It is unclear whether this is the intended meaning, because the fragments of claims 9 and 10, for example, appear to encompass only part of SEQ ID NO:33.

16. Claims 1, 13-15 and dependent claims 17-19 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for the scope of claim 16, does not reasonably enable the range of polypeptides encompassed in the instant claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. § 112, first paragraph, have been clarified in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the instantly claimed invention. First, the specification teaches at Example 5 a purified polypeptide having an apparent molecular weight of approximately 23 kD and an N-terminal sequence consisting of SEQ ID NO:13. No other polypeptides which specifically bind the Flt4 receptor are taught. Second, claim 1 encompasses all proteins that may interact with the Flt4 receptor, and claim 13 encompasses all 23 kD fragments of any portion of all of these polypeptides. Claim 15 encompasses all 23 kD polypeptides comprising the 17 amino acids of SEQ ID NO:13 which may be present at any region of these polypeptides. This claim encompasses about 34 polypeptides from the protein of

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standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

The art can define high affinity and non-specific binding to receptors; however, the instant inventions encompasses numerous polypeptides which would be expected to have affinities for the receptor anywhere between high affinity to non-specific binding. Intermediate affinities are not definable without a standard of affinity for "specific" binding, and hence it is impossible to determine whether such compounds would be included within the bounds of the claims.

14. Claim 10 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claim is presumably drawn to a polypeptide but refers to SEQ ID NO:32, which is a nucleic acid sequence.

Furthermore, the term "approximately" in claim 10 is a relative term which renders the claim indefinite. The term "approximately" is not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention.

In the instant case, it is not clear whether "approximately" is used in reference to the length or the composition of the polypeptide. In both cases the bounds of the claim cannot be determined without a standard of reference. It is not clear, for example, whether the claim encompasses polypeptides larger than the first 180 amino acids such as the precursor to the protein of SEQ ID NO:32.

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#### *Claim Objections*

11. Claim 17 is objected to under 37 C.F.R. § 1.75(c) for not further limiting the subject matter of claim 1.

Specifically, the instant claim does not recite further structural limitations to the polypeptide of claim 1; the particular source of the polypeptide is *de minimus*.

12. Claim 14 is objected to under 37 C.F.R. § 1.75(c) for not further limiting the subject matter of claim 13.

Claim 14 recites the functional limitation of stimulating Flt4 phosphorylation, which is a functional limitation not enabled by the teachings of the specification (M.P.E.P. § 2173.05(g)). The specification teaches a polypeptide of 23 kD that stimulates Flt4 phosphorylation at Figure 5. The specification does not teach necessary or sufficient structures of the 23 kD protein that promote phosphorylation, nor could the skilled artisan have predicted what these structures would be from the state of the art at the time of the invention. Claim 14 does not further limit claim 13, because the limitation of promoting phosphorylation in the instant claim does not further limit the structure of the polypeptide of claim 13.

#### *Claim Rejections - 35 USC § 112*

13. Claims 1 and 8, and dependent claims 9, 10, 13-19 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The term "specifically" in claims 1 and 8 is a relative term which renders the claim indefinite. The term "specifically" is not defined by the claim, the specification does not provide a

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#### *Oath/Declaration*

7. The oath or declaration is defective. A new oath or declaration in compliance with 37 C.F.R. § 1.67(a) identifying this application by its Serial Number and filing date is required. See M.P.E.P. §§ 602.01 and 602.02.

The oath or declaration is defective because:

It does not state that the person making the oath or declaration in a continuation-in-part application filed under the conditions specified in 35 U.S.C. § 120 which discloses and claims subject matter in addition to that disclosed in the prior copending application, acknowledges the duty to disclose material information as defined in 37 C.F.R. § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

The Examiner notes that the supplemental declaration was to be included in Paper No. 10, but was either misplaced or omitted during transmittal.

#### *Drawings*

8. This application has been filed with informal drawings which are acceptable for examination purposes only. Pursuant to a change in Office policy effective 25 April 1996, *formal drawings will be required at the time allowable subject matter is first indicated.*

Please note the Examiner incorrectly submitted the PTO 948 to the Draftman for review. Review will be appropriate upon submission of formal drawings; the PTO 948 will not be attached to the Office action in Paper No. 11.

#### *Specification*

9. The disclosure is objected to because of the following informalities: 3' deletions of the Flt4 ligand are taught at p. 27, line 24, but deletions occurring at the end of a protein are preferably termed "C-terminal" or "carboxy terminal" deletions. "3'" is preferred for describing nucleic acids only. Appropriate correction is required.

10. The lengthy specification has not been checked to the extent necessary to determine the presence of all possible minor errors. Applicant's cooperation is requested in correcting any errors of which applicant may become aware in the specification.

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*Priority*

6. If the filing date of an application to which priority is claimed under 35 U.S.C. § 120 is needed to overcome an intervening reference, there is a need for the Office to make a determination as to whether the requirement of 35 U.S.C. § 120, that the earlier application discloses the invention of the second application in the manner provided by the first paragraph of 35 U.S.C. 112, is met and whether a substantial portion of all of the earlier application is repeated in the second application in a continuation-in-part situation (M.P.E.P. § 201.08).

Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. § 112, first paragraph, have been clarified in *Ex parte Forman*, 230 USPQ 546 (BPAI 1986). As summarized in *Ex parte Forman*, the following considerations are relevant to the analysis of enablement for the claimed invention of an Flt4 ligand in application Serial No. 08/340011. First, the instant specification teaches at p.7, line38 through p.8, line 1, that the Flt receptor *may* prove useful in obtaining a ligand that binds to it, but no such method is taught. There are no other teachings of the ligand. Second, no working examples are given that teach a ligand to the receptor. Third, the skilled artisan at the time of the instant invention had no means of predicting which compounds would be ligands for the receptor, the requisite degree of affinity for specific binding to the receptor was unknown and could not be predicted, and success in using the receptor to obtain the ligand could not be predicted. Additionally, the specification does not teach that the expressed receptor is functionally active, and the skilled artisan cannot predict the success of any method of obtaining a ligand to a receptor that is not biologically active. Thus, undue experimentation would be required to make the instant ligand. Consequently, no claims are afforded priority to application Serial No. 08/340011.

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patent files is fundamental in establishing patentability and underlies this *prima facie* showing of burden. Prior art searches on proteins, nucleic acids, or antibodies must address separate issues of patentability in each case:

(3) This Action, while cognizant of the financial resources expended on prosecution, respectfully submits that consideration of monetary burden on Office or applicant is not recognized as a ground for establishing a burden to examine or a lack thereof.

The requirement is still deemed proper and is therefore made FINAL.

2. Claims 3, 4, 6 (each amended), 5, 7, and 11 are withdrawn from further consideration by the examiner, 37 C.F.R. § 1.142(b), as being drawn to non-elected inventions, the requirement having been traversed in Paper No. 9.

3. This application contains claims 3, 4, 6 (each amended), 5, 7, and 11 drawn to inventions non-elected with traverse in Paper No. 9. A complete response to the final rejection must include cancellation of non-elected claims or other appropriate action (37 C.F.R. § 1.144; M.P.E.P. § 821.01).

4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

5. Pursuant to the Preliminary Amendment in Paper No. 10, claims 1 (amended), 2 (amended), 8 (amended), 9 (amended), 10, 12 (amended), and newly added claims 13-19 are pending in the instant application.

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### Part III DETAILED ACTION

#### *Election/Restriction*

I. Applicant's election with traverse of Group I (claims 1, 2, 8-10, and 12) in Paper No. 9 is acknowledged. The traversal is on the grounds that (1) the Restriction Requirement has not demonstrated that the inventions satisfy the "independent and distinct" provision of 35 U.S.C. § 121, (2) the Restriction Requirement has not demonstrated burden, and (3) withdrawal of the requirement for restriction will conserve resources of the Office and applicant. This is not found persuasive because of the following reasons:

(1) This Action respectfully refers to M.P.E.P. § 802.01, which states:

The law has long been established that dependent inventions . . . may be properly divided if they are, in fact, "distinct" inventions, even though dependent.

Further elaboration of "distinct" is provided in M.P.E.P. § 802.01. The Requirement for Restriction has correctly defined the distinctness of the inventions as required by 35 U.S.C. § 121 for the reasons made of record in Paper No. 9. Restriction would be improper if prior art reading on one invention would make the other obvious. Prior art anticipating a protein does not necessarily render the encoding DNA obvious because of the redundancy of the genetic code (M.P.E.P. § 2144.09, p. 104). Prior art anticipating a protein does not necessarily make the antibody obvious, because motivation must be present in the prior art to obtain that antibody (M.P.E.P. § 2143.01), and because antibodies may be of different varieties, which has raised different issues regarding obviousness in the case law.

(2) In reference to M.P.E.P. § 803, this Action respectfully refers to M.P.E.P. § 808.02, wherein it states that *prima facie* burden is correctly established by demonstration of separate classification. A thorough search of the relevant prior art in both electronic databases and in the



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

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APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
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1. 31. 12/95 611743

K 28110/32863

EXAMINER
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CRISTOPHER B

ART UNIT	PAPER NUMBER
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11

DATE MAILED: 12/14

09/10/96

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

OFFICE ACTION SUMMARY

- ☒ Responsive to communication(s) filed on 7/26/96
- ☐ This action is FINAL.
- ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 D.C. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire 3 month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

- ☒ Claim(s) 1-19 is/are pending in the application.
- Of the above, claim(s) 3-7, 11 is/are withdrawn from consideration.
- ☒ Claim(s) 2, 12 is/are allowed.
- ☒ Claim(s) 1, 8-10, 13-19 is/are rejected.
- ☒ Claim(s) 14, 17 is/are objected to.
- ☐ Claims are subject to restriction or election requirement.

Application Papers

- ☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.
- ☐ The drawing(s) filed on \_\_\_\_\_ is/are objected to by the Examiner.
- ☐ The proposed drawing correction, filed on \_\_\_\_\_ is ☐ approved ☐ disapproved.
- ☐ The specification is objected to by the Examiner.
- ☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

- ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).
- ☐ All ☐ Some\* ☐ None of the CERTIFIED copies of the priority documents have been
- ☐ received.
- ☐ received in Application No. (Series Code/Serial Number) \_\_\_\_\_.
- ☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\*Certified copies not received: \_\_\_\_\_

- ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

- ☒ Notice of Reference Cited, PTO-892
- ☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). 5
- ☐ Interview Summary, PTO-413
- ☐ Notice of Draftsperson's Patent Drawing Review, PTO-948
- ☐ Notice of Informal Patent Application, PTO-152

- SEE OFFICE ACTION ON THE FOLLOWING PAGES -



19. A polypeptide according to claim 1 further comprising a detectable label. —

**REMARKS**

The specification has been amended herein to claim priority from an earlier-filed U.S. application. This amendment is accompanied by a supplemental inventors' declaration which acknowledges this priority claim.

The specification has been amended at page 26 to make reference to a Budapest Treaty biological deposit of a vector of the invention that is described at p. 26, lines 5-8. Such an amendment does not constitute new matter. See *In re Lundak*, 773 F.2d 1216, 1223, 227 U.S.P.Q. 90, 96 (Fed. Cir. 1985). Claim 6 has been similarly amended to claim this vector.

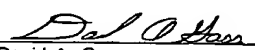
The amendment to claim 9 finds support throughout the specification, including at p. 18, lines 26-30.

New claims 13-18 find support throughout the specification, including in Example 5, and particularly at p. 18, line 16, to p. 19, line 19.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Dated: August 12, 1996

  
David A. Gass  
Registration No. 38,153  
6300 Sears Tower  
233 S. Wacker Drive  
Chicago, Illinois 60606  
Telephone: (312) 474-6300

12. (Amended) A pharmaceutical composition comprising a [peptide]  
polypeptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant,  
or carrier.

-13. A polypeptide according to claim 1 having an apparent  
molecular weight of approximately 23 kD as assessed by SDS-PAGE under  
reducing conditions.

14. A polypeptide according to claim 13 which is capable of  
stimulating Flt4 phosphorylation in mammalian cells expressing Flt4 receptor  
tyrosine kinase.

15. A purified and isolated polypeptide according to claim 14, said  
polypeptide comprising an amino acid sequence set forth in SEQ ID NO: 13.

16. A purified and isolated polypeptide according to claim 13,  
wherein amino terminal amino acids 2 through 18 of said polypeptide have an  
amino acid sequence corresponding to amino acids 2 through 18 set forth in SEQ  
ID NO: 13.

17. A purified and isolated polypeptide according to claim 1, said  
polypeptide being purifiable from conditioned media from a PC-3 prostatic  
adenocarcinoma cell line, said cell line having ATCC CRL No. 1435.

18. A conditioned medium comprising a polypeptide according to  
claim 1.

In the claims:

Please amend claims 1-4, 6, 8-9, and 12, and add new claims 13-19 as shown below:

1. (Amended) A purified and isolated polypeptide [peptide] which specifically binds to the Flt4 receptor tyrosine kinase.

2. (Amended) A purified and isolated polypeptide comprising an [peptide having the] amino acid sequence shown in SEQ ID NO: 33.

3. (Amended) A purified and isolated nucleic acid encoding the purified and isolated polypeptide [peptide] according to claim 2.

4. (Amended) The nucleic acid according to claim 3 having [the] a nucleotide sequence shown in SEQ ID NO: 32.

6. (Amended) The vector according to claim 5, wherein said vector is plasmid [pFlt4] pFlt4-L, deposited as ATCC accession No. 97231.

8. (Amended) A fragment of the purified and isolated [peptide] polypeptide according to claim 2 which is capable of specifically binding to an Flt4 receptor tyrosine kinase.

9. (Amended) The fragment according to claim 8 having an apparent molecular weight of approximately 23 kD as assessed by SDS-PAGE under reducing conditions.

AUG 12 1996 12:34PM

MARSHALL O'TOOLE

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PATENT  
28113/32863

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Alitalo et al.

Serial No: 08/510,133

Filed: August 1, 1995

Title: RECEPTOR LIGAND

Group Art Unit: 1814

Examiner: Lathrop, B.

I hereby certify that this paper is  
being deposited with the United  
States Postal Service with sufficient  
postage as first class mail in an  
envelope addressed to: Assistant  
Commissioner for Patents,  
Washington, D.C., 20231 on this  
date:

Date: August 12, 1996

David A. Gass  
David A. Gass  
Registration No. 38,153  
Attorney for Applicants

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

The Applicants respectfully request entry of this Preliminary  
Amendment prior to examination of the above-identified application on the merits  
by the Patent and Trademark Office.

AMENDMENTS

In the Specification:

At page 1, line 2, please insert the following priority claim:

-- This application is a continuation-in-part of U.S. Patent Application Serial No.  
08/340,011, filed November 14, 1994. --

At page 26, line 10, please delete "pFLT4" and substitute therefor --  
pFit4-L--.

At page 26, line 12, please delete "\_\_\_\_\_" and substitute  
therefor --97231--.



PATENT

THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Alitalo et al.

Serial No. 08/510,133

Filed: August 1, 1995

For: RECEPTOR LIGAND

Art Unit: 1814

Examiner: Lathrop, B.

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mail, postage prepaid, in an  
envelope addressed to: Assistant  
Commissioner for Patents  
Washington, D.C. 20231, on this  
date:

Dated: July 24, 1996

David A. Gass  
David A. Gass

**ELECTION WITH TRAVERSE IN RESPONSE TO RESTRICTION REQUIREMENT**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

In an official communication dated May 29, 1996, the U.S. Patent and Trademark Office issued a restriction requirement in the above-identified patent application, and set a 30 day period for response. This response to the restriction requirement has been timely filed with a petition for one month extension of time and petition fee. Reconsideration of the restriction requirement is respectfully requested in light of the following remarks.

- I. The restriction requirement should be withdrawn because the Examiner has not demonstrated that the "independent and distinct" provisions of 35 U.S.C. §121 have been satisfied.

The Examiner has required restriction of the claims of the application for examination purposes, citing 35 U.S.C. §121. The Applicants traverse the restriction requirement. Reconsideration is requested.

The provisions of 35 U.S.C. §121 state, "If two or more independent and distinct inventions are claimed in one application, the Commissioner may require the application to be restricted to one of the inventions." (Emphasis added.) The Examiner has failed to demonstrate or assert that the claims divided into Groups I, II, and III are "independent" from each other.

According to the Patent Office's own procedure manual, "The term 'independent' (i.e., not dependent) means that there is no disclosed relationship between the two or more subjects disclosed, that is, they are unconnected in

design, operation or effect . . . ." See M.P.E.P. §802.01 (emphasis added). In the restriction requirement, the Patent Office has acknowledged that the allegedly distinct three groups of claims are related. For example, the Patent Office states that the "protein of Group I" and "the nucleic acid of Group II" are "related as the nucleic acid encodes the Flt4 ligand." (Restriction Requirement at p. 2.) Thus, a nucleic acid which encodes a protein is connected in operation and effect to the encoded protein. The lack of "independence" between Groups I and II also is manifest from the claims themselves. For example, claims 3-7 of Group II depend from claim 2 of Group I. Because the claims of Groups I and II are related, the subject matters of the two groups are not "independent" as required by 35 U.S.C. §121, and the restriction requirement was improper as between these two groups of claims.

Similarly, the claim of Group III, "drawn to an antibody," is not "independent" of the claims of Group I, "drawn to an Flt4 ligand." For example, an antibody to a protein is connected to the protein in design, operation, and effect because one skilled in the art uses the protein (or portions thereof) as an antigen to generate the antibody. The antibody, in turn, reacts immunologically with the protein with a great degree of specificity. Because the claims of Groups I and III are related, the groups are not "independent."

For the reasons stated above, the Examiner has not demonstrated that the divided Groups of claims are "independent" from each other as required by 35 U.S.C. §121. Thus, the Examiner has failed to meet the burden for imposing a restriction requirement.

- II. The restriction requirement should be withdrawn because the Examiner has not demonstrated a serious burden will result if restriction is not required, and because withdrawal will conserve resources of the Patent and Trademark Office and the Applicants.

Assuming *arguendo* that the claims as grouped by the Examiner are "independent" and "distinct," the restriction requirement is nonetheless improper, because the Examiner has failed to demonstrate that "a serious burden" will result if restriction is not required. See M.P.E.P. §803 ("If the search and examination of an entire application can be made without serious burden, the examiner must examine it on the merits, even though it includes claims to distinct or independent

inventions.") No such burden has been alleged in the Office action.<sup>1</sup> In fact, removal of the restriction requirement will conserve the resources of the Patent and Trademark Office and the Applicants, by minimizing the number of similar searches performed by the Patent Office examiners and by reducing the filing fees and prosecution costs of the Applicants.

For example, the Patent Office has acknowledged a relationship between a protein and the nucleic acids which encode it. When conducting a thorough prior art search for the purified and isolated polypeptide of the invention (Group I), the Patent Office will undoubtedly wish to search the art related to nucleic acids (Group II) which encode proteins, due to the universal nature of the genetic code. Moreover, because it is a common practice in the art to report nucleic acid and deduced amino acid sequences simultaneously, these arts are highly co-extensive. The burden on the Patent Office in searching both the nucleic acid and amino acid arts, if any, will not be serious.

Similarly, it is a common practice in the art to characterize antibodies with reference to the antigen which the antibodies recognize. Therefore, when conducting a thorough prior art search related to the antibody claim 11 (Group III), the Patent Office will undoubtedly search the art related to proteins, i.e., the art that it will search for the claims of Group I.

Thus, a thorough examination of the claims in any particular group will involve a search of the art pertinent to the subject matter of the claims of the other groups. Hence, no serious burden will result if restriction is not required in this case. However, if the restriction requirement is maintained, the Applicants will incur additional prosecution costs associated with filing and prosecuting divisional applications, and the Patent and Trademark Office will be required to perform a duplicative search of the same prior art. Thus, withdrawal of the restriction requirement will conserve the resources of the Patent and Trademark Office and of the Applicants without causing a serious burden on the Patent and Trademark Office.

For these reasons, the restriction requirement imposed in the Office action should be withdrawn.

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<sup>1</sup> The different Patent and Trademark Office classifications of the claims in Groups I, II, and III are not an indication of independence or distinction under § 121. Nor are the classifications an indication that an undue burden exists on the Examiner. Such classifications merely serve as a convenient search tool.

III. The Applicants Elect Claims 1, 2, 8-10, and 12 (Group I) with traverse.

In response to the restriction requirement, the Applicants hereby elect Group I (claims 1, 2, 8-10, and 12), with traverse.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Date: July 24, 1996

By:

David A. Gass  
David A. Gass  
Reg. No: 38,153





UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
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1812/0329

28113/32869	
EXAMINER	
LATHROP, E	
ART UNIT	PAPER NUMBER

1812/0329  
MURRAY  
4300 PEARSON COURT  
733 SOUTH WICKER DRIVE  
CHICAGO IL 60606-6402

1814  
DATE MAILED: 05/29/96

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on \_\_\_\_\_ ☐ This action is made final.  
The statutory period for response to this action is set to expire \_\_\_\_\_ month(s), 30 days from the date of this letter.  
Failure to respond within this period for response will cause the application to become abandoned. 35 U.S.C. 133

PART I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- |   |   |
|---|---|
| 1. <input type="checkbox"/> Notice of References Cited by Examiner, PTO-892.        | 2. <input type="checkbox"/> Notice of Draftsman's Patent Drawing Review, PTO-948. |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449.             | 4. <input type="checkbox"/> Notice of Informal Patent Application, PTO-152.       |
| 5. <input type="checkbox"/> Information on how to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____   |

PART II SUMMARY OF ACTION

1. ☒ Claims 1-12 are pending in the application.  
Of the above, claims \_\_\_\_\_ are withdrawn from consideration.
2. ☐ Claims \_\_\_\_\_ have been cancelled.
3. ☐ Claims \_\_\_\_\_ are allowed.
4. ☐ Claims \_\_\_\_\_ are rejected.
5. ☐ Claims \_\_\_\_\_ are objected to.
6. ☒ Claims 1-12 are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on \_\_\_\_\_. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed \_\_\_\_\_, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other \_\_\_\_\_

EXAMINER'S ACTION

### Part III DETAILED ACTION

#### *Election/Restriction*

1. Restriction to one of the following inventions is required under 35 U.S.C. 121:  
Group I. Claims 1, 2, 8-10, and 12, drawn to an Flt4 ligand and a pharmaceutical composition comprising the same, classified in Class 514, subclass 2.  
Group II. Claims 3-7, drawn to nucleic acid encoding an Flt4 ligand, classified in Class 536, subclass 23.5.  
Group III. Claim 11, drawn to an antibody reactive against an Flt4 ligand, classified in Class 530, subclass 387.9.

The inventions are distinct, each from the other, because of the following reasons:

2. The protein of Group I is a patentably distinct chemical species from the nucleic acid of Group II, although related as the nucleic acid encodes the Flt4 ligand. The ligand can be made without recourse to the nucleic acid by standard methods of biochemical purification from tissues, and the nucleic acid has separate utility as a probe for detection of complementary genomic sequences, for example.
3. The protein of Group I is a patentably distinct chemical species from the antibody of Group III, although the antibody is directed to the Flt4 ligand. The ligand can be made without recourse to the antibody by standard methods of biochemical purification from tissues, and the antibody has a separate utility as a probe for screening an expression library, for example.
4. The antibody of Group III is a patentably distinct chemical species from the nucleic acid of Group II, because the antibody can be raised to ligand purified without recourse to the nucleic acid, and the nucleic acid has separate utility as a probe for detection of complementary genomic sequences, for example.
5. Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, and because the search for each group of inventions is not coextensive with the searches of the inventions of the other groups, restriction for examination purposes as indicated is proper.
6. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 C.F.R. § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a diligently-filed petition under 37 C.F.R. § 1.48(b) and by the fee required under 37 C.F.R. § 1.17(h).

Serial Number: 08/510,133  
Art Unit: 1814

-3-


7. A telephone call was made to David Gass on 16 May 1996 to request an oral election to the above restriction, but the call did not result in an election being made.

8. Any inquiry concerning this communication from the examiner should be directed to Brian Lathrop whose telephone number is (703) 305-5679. The examiner can normally be reached Monday through Friday from 8:30 AM to 5:00 PM.

The examiner will attempt to respond to voice mail messages within 24 hours. Alternately, the examiners's supervisor, Robert A. Wax, can be reached at (703) 308-4216. The FAX number for Group 1814 is (703) 305-7401.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

Brian K. Lathrop, Ph.D.  
AU 1814

  
ROBERT A. WAX  
SUPERVISORY PATENT EXAMINER  
GROUP 180



3-115 (Gass)  
1814  
#8888  
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):

Alitalo et al.

Serial No: 08/510,133

Filed: August 1, 1995


Title: Receptor Ligand

Group Art Unit: 1814

Examiner: Lathrop, B.

**CERTIFICATE OF MAILING**  
**(37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on July 24, 1996, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

  
David A. Gass

**PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Applicants hereby petition pursuant to 37 CFR 1.136(a) for a one month extension of time. Attached is a check in the amount of \$110.00 in payment of the petition fee.

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 to Deposit Account No. 13-2855. A copy of this Petition is enclosed.


Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

Date: 8-1-96

By:

  
David A. Gass  
Reg. No: 38,153  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

Also enclosed is a copy of the Notice together with our check in the amount of \$130.00 in payment of the fee.

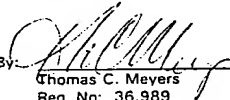
The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required under 37 CFR 1.16 or 1.17 to Deposit Account No. 13-2855. A copy of this request is enclosed.

Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By

  
Thomas C. Meyers  
Reg. No: 36,989

December 19, 1995

**PATENT**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Applicants:	)	
Kari Alitalo and Vladomir Joukov	)	Title: Receptor Ligand
Serial No: 08/510,133	)	
Filed: August 1, 1995	)	

**TRANSMITTAL OF EXECUTED DECLARATION**

***Assistant Commissioner for Patents  
Washington, D.C. 20231***

***Attention: Application Branch***

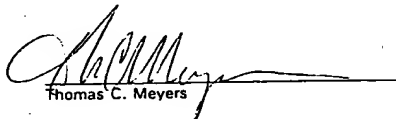
Sir:

Submitted herewith is an executed Declaration for filing in the above-identified application, in response to the Notice to File Missing Parts issued by the Patent and Trademark Office on November 20, 1995.

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**CERTIFICATE OF MAILING (37 CFR 1.8)**

I hereby certify that this paper and the documents referred to as enclosed therewith are being deposited with the United States Postal Service as first class mail, postage prepaid, on December 19, 1995 in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231.

  
Thomas C. Meyers



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
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03/510,133 08/01/95 ALITALO

K 28113/32863

0282/1120

MARSHALL O'TOOLE GERSTEIN  
MURRAY AND BORUN  
6300 SEARS TOWER  
233 SOUTH WACKER DRIVE  
CHICAGO IL 60606-6402

DATE MAILED: 0000

**NOTICE TO FILE MISSING PARTS OF APPLICATION 11/20/95**  
**FILING DATE GRANTED**

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130 for large entities or \$ 65 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 130.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$ \_\_\_\_\_ to complete the basic filing fee.
  2. ☐ Additional claim fees of \$ \_\_\_\_\_ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
  3. ☒ The oath or declaration:  
☒ is missing.  
☐ does not cover the newly submitted items.
- An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.
4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
  5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
  6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:  
\_\_\_\_\_  
An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
  7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ \_\_\_\_\_ under 37 CFR 1.17(k), unless this fee has already been paid.
  8. ☐ A \$ \_\_\_\_\_ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
  9. ☐ Your filing receipt was mailed in error because your check was returned without payment.
  10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
  11. ☐ Other.

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

**A copy of this notice MUST be returned with the response.**



136-105-AA  
UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
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136-105-AA

2011/07/26/00

REYNOLD, GORDON L. BERNSTEIN  
JENNIFER ANN BERNSTEIN  
2000 N. WILSON ROAD  
200 SOUTH BAKER DRIVE  
CHICAGO, IL 60604-1100

02/27/12

DATE MAILED: 09/10

**NOTICE TO FILE MISSING PARTS OF APPLICATION  
FILING DATE GRANTED**

11/20/05

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$130 for large entities or \$65 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$130.

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- ☐ The statutory basic filing fee is: ☐ missing ☐ insufficient. Applicant as a ☐ large entity ☐ small entity, must submit \$\_\_\_\_\_ to complete the basic filing fee.
- ☐ Additional claim fees of \$\_\_\_\_\_ as a ☐ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fee or cancel the additional claims for which fees are due.
- ☒ The oath or declaration:  
☒ is missing.  
☐ does not cover the newly submitted items.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.

- ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:  
\_\_\_\_\_. An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
- ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$\_\_\_\_\_ under 37 CFR 1.17(k), unless this fee has already been paid.
- ☐ A \$\_\_\_\_\_ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
- ☐ Your filing receipt was mailed in error because your check was returned without payment.
- ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.
- ☐ Other: 08/10/13 1 105 130.00 CK

Direct the response to Box Missing Part and refer any questions to the Customer Service Center at (703) 308-1202.

**A copy of this notice MUST be returned with the response.**





Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/32863	Serial No. 08/510,133
<b>INFORMATION DISCLOSURE STATEMENT</b> (Use several sheets if necessary)		Applicant Alitalo, <i>et al.</i>	
		Filing Date 8/1/95	Group

20

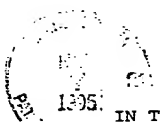
OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
PKL	C1	Ausprunk, <i>et al.</i> , "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis", <i>Microvascular Research</i> , 14:53-65 (1977).
PKL	C2	Breier, <i>et al.</i> , "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation", <i>Development</i> , 114:521-532 (1992).
PKL	C3	Dignam, <i>et al.</i> , "Balbiani ring 3 in <i>Chironomus tentans</i> encodes a 185-kDa secretory protein which is synthesized throughout the fourth larval instar", <i>Gene</i> , 88:133-140 (1990).
PKL	C4	Don, <i>et al.</i> , "'Touchdown' PCR to circumvent spurious priming during gene amplification", <i>Nucleic Acids Research</i> , 19(14):4008 (1991).
PKL	C5	Folkman, <i>et al.</i> , "Angiogenesis", <i>The Journal of Biological Chemistry</i> , 267(15):10931-10934 (1992).
PKL	C6	Kozak, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs", <i>Nucleic Acids Research</i> , 15(20):8125-8148 (1987).
PKL	C7	Mäkelä, <i>et al.</i> , "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector", <i>Gene</i> , 118:293-294 (1992).
PKL	C8	Pajusola, <i>et al.</i> , "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-like Loops and Is Expressed in Multiple Human Tissues and Cell Lines", <i>Cancer Research</i> , 52:5738-5743 (1992).
PKL	C9	Pajusola, <i>et al.</i> , "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts", <i>Oncogene</i> , 8:2931-2937 (1993).
PKL	C10	Risau, <i>et al.</i> , "Changes in the Vascular Extracellular Matrix during Embryonic Vasculogenesis and Angiogenesis", <i>Developmental Biology</i> , 125:441-450 (1988).
PKL	C11	Saksela, <i>et al.</i> , "Cell-Associated Plasminogen Activation: Regulation and Physiological Functions", <i>Annu. Rev. Cell. Biol.</i> , 4:93-126 (1988).
PKL	C12	Tessier, <i>et al.</i> , "Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide", <i>Gene</i> , 98:177-183 (1991).
PKL	C13	van der Geer, <i>et al.</i> , "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways", <i>Annu. Rev. Cell. Biol.</i> , 10:251-337 (1994).

EXAMINER <i>Kir M. L. H. H. H.</i>	DATE CONSIDERED 8/28/96
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	

Form PTO-1449 (Modified)	U.S. Department of Commerce Patent and Trademark Office	Atty. Docket No. 28113/32863	Serial No. 08/510,133
<b>INFORMATION DISCLOSURE STATEMENT</b> <i>(Use several sheets if necessary)</i>		Applicant Alitalo, et al.	
		Filing Date 8/1/95	Group

OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Pages, etc.)		
8/21	C1	Ausprunk, et al., "Migration and Proliferation of Endothelial Cells in Preformed and Newly Formed Blood Vessels during Tumor Angiogenesis", <i>Microvascular Research</i> , 14:53-65 (1977).
8/21	C2	Breier, et al., "Expression of vascular endothelial growth factor during embryonic angiogenesis and endothelial cell differentiation", <i>Development</i> , 114:521-532 (1992).
8/21	C3	Dignam, et al., "Babian ring 3 in <i>Chironomus tentans</i> encodes a 185-kDa secretory protein which is synthesized throughout the fourth larval instar", <i>Gene</i> , 88:133-140 (1990).
8/21	C4	Don, et al., "'Touchdown' PCR to circumvent spurious priming during gene amplification", <i>Nucleic Acids Research</i> , 19(14):4008 (1991).
8/21	C5	Folkman, et al., "Angiogenesis", <i>The Journal of Biological Chemistry</i> , 267(16):10931-10934 (1992).
8/21	C6	Kozak, "An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs", <i>Nucleic Acids Research</i> , 15(20):8125-8148 (1987).
8/21	C7	Mäkelä, et al., "Plasmid pLTRpoly: A Versatile High-Efficiency Mammalian Expression Vector", <i>Gene</i> , 118:293-294 (1992).
8/21	C8	Pajusola, et al., "FLT4 Receptor Tyrosine Kinase Contains Seven Immunoglobulin-like Loops and Is Expressed in Multiple Human Tissues and Cell Lines", <i>Cancer Research</i> , 52:5738-5743 (1992).
8/21	C9	Pajusola, et al., "Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts", <i>Oncogene</i> , 8:2931-2937 (1993).
8/21	C10	Risau, et al., "Changes in the Vascular Extracellular Matrix during Embryonic Vasculogenesis and Angiogenesis", <i>Developmental Biology</i> , 125:441-450 (1988).
8/21	C11	Saksela, et al., "Cell-Associated Plasminogen Activation: Regulation and Physiological Functions", <i>Annu. Rev. Cell. Biol.</i> , 4:93-126 (1988).
8/21	C12	Tessier, et al., "Enhanced secretion from insect cells of a foreign protein fused to the honeybee melittin signal peptide", <i>Gene</i> , 98:177-183 (1991).
8/21	C13	van der Geer, et al., "Receptor Protein-Tyrosine Kinases and Their Signal Transduction Pathways", <i>Annu. Rev. Cell. Biol.</i> , 10:251-337 (1994).

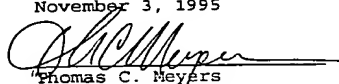
EXAMINER F. J. L. L. L.	DATE CONSIDERED 8/28/96
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.	



600

File #6  
BCC MW  
PATENT 51-90

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	)	I hereby certify that this
	)	paper is being deposited
Alitalo, et al.	)	with the United States
	)	Postal Service as first
Serial No.: 08/510,133	)	class mail, postage
	)	prepaid, in an envelope
	)	addressed to:
Filed: August 1, 1995	)	Commissioner of Patents
	)	and Trademarks,
For: "Receptor Ligand"	)	Washington, DC 20231 on
	)	this date:
Group Art Unit: TBD	)	November 3, 1995
Examiner: TBD	)	
	)	
	)	Thomas C. Meyers
	)	Reg. No. 36,989
	)	Attorney for Applicants

INFORMATION DISCLOSURE STATEMENT


Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

In compliance with 37 C.F.R. §1.97 and the continuing duty of disclosure under 37 C.F.R. §1.56, the attached form PTO-1449 and the items of information cited therein are hereby submitted by Applicants for consideration in connection with the above-identified patent application. This statement and the accompanying items of information, including form PTO-1449, are being submitted within three months of the filing of the above-identified patent application. Accordingly, it is submitted that no fee is due in this matter under 37 C.F.R. §1.97(b). However, if it is determined that any appropriate fee is due, please charge Deposit Account No. 13-2855. A duplicate of this paper is enclosed.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By   
Thomas C. Meyers  
Reg. No. 36,989  
Attorneys for Applicants  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

November 3, 1995

PAGE: 5

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/510,133

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210 (D) TOPOLOGY: linear

211  
212 (ii) MOLECULE TYPE: DNA (genomic)

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20

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230 1 5 10 15

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232 Leu Lys  
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234

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247 GCAGARGARA CNATHAA

17

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255 (C) STRANDEDNESS: single  
256 (D) TOPOLOGY: linear

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258 (ii) MOLECULE TYPE: DNA (genomic)

PAGE: 4

RAW SEQUENCE LISTING  
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154   1                               5               10               15
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PAGE: 3

RAW SEQUENCE LISTING  
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131 (B) TYPE: nucleic acid
132 (C) STRANDEDNESS: single
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17

34

PAGE: 2

RAW SEQUENCE LISTING  
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TEAM 8

PAGE: 1

RAW SEQUENCE LISTING  
PATENT APPLICATION US/08/510,133

DATE: 10/04/95  
TIME: 11:46:31

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This Raw Listing contains the General  
Information Section and up to the first 5 pages.

SEQUENCE LISTING

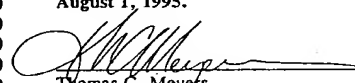
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3 (1) General Information:  
4 (i) APPLICANT: Alitalo, Kari  
5 Joukov, Vladomir  
6  
7 (ii) TITLE OF INVENTION: Receptor Ligand  
8  
9 (iii) NUMBER OF SEQUENCES: 33  
10  
11 (iv) CORRESPONDENCE ADDRESS:  
12 (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun  
13 (B) STREET: 6300 Sears Tower, 233 South Wacker Drive  
14 (C) CITY: Chicago  
15 (D) STATE: Illinois  
16 (E) COUNTRY: United States of America  
17 (F) ZIP: 60606-6402  
18  
19 (v) COMPUTER READABLE FORM:  
20 (A) MEDIUM TYPE: Floppy disk  
21 (B) COMPUTER: IBM PC compatible  
22 (C) OPERATING SYSTEM: PC-DOS/MS-DOS  
23 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25  
24  
25 (vi) CURRENT APPLICATION DATA:  
26 (A) APPLICATION NUMBER:  
27 (B) FILING DATE:  
28 (C) CLASSIFICATION:  
29  
30 (viii) ATTORNEY/AGENT INFORMATION:  
31 (A) NAME: Meyers, Thomas C.  
32 (B) REGISTRATION NUMBER: 36,989  
33 (C) REFERENCE/DOCKET NUMBER: 28113/32863  
34  
35 (ix) TELECOMMUNICATION INFORMATION:  
36 (A) TELEPHONE: 312/474-6300  
37 (B) TELEFAX: 312/474-0448  
38 (C) TELEX: 25-3856  
39  
40 (2) INFORMATION FOR SEQ ID NO:1:  
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43 (A) LENGTH: 20 base pairs  
44 (B) TYPE: nucleic acid  
45 (C) STRANDEDNESS: single  
46

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08/51013

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	)	I hereby certify that this paper is
	)	being deposited with the United
Alitalo, <i>et al.</i>	)	States Postal Service, in an envelope
	)	addressed to: Assistant
	)	Commissioner for Patents,
Serial No.: TBD	)	Washington, DC 20231 utilizing the
	)	"Express Mail Post Office to
	)	Addressee" service of the United
Filed: Herewith	)	States Postal Service under Mailing
	)	Label No. EG 473 138 672 US on
	)	August 1, 1995.
For: "Receptor Ligand"	)	
	)	
	)	Thomas C. Meyers
	)	Reg. No. 36,989
	)	Attorney for Applicants

TRANSMITTAL OF SEQUENCE LISTING AND  
STATEMENT UNDER 37 C.F.R. §1.821(f)

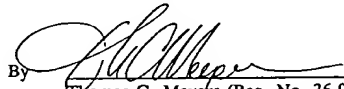
Assistant Commissioner for Patents  
Washington, DC 20231

Sir:

I hereby state that the content of the paper and computer readable  
copies of the Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(c)  
and (e), respectively, are the same.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN

By   
Thomas C. Meyers (Reg. No. 36,989)  
Attorneys for Applicants  
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233 South Wacker Drive  
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(312) 474-6300

Secord Joint Inventor, if any Vladimir Joukov <i>2-10</i>	Citizenship Finland
Residence Address - Street Topeliuksenkatu 32G8	Post Office Address - Street Same
City (Zip) 00290 Helsinki <i>FIK</i>	City (Zip) Same
State or Country FINLAND	State or Country Same
Date 30.11.1945	Signature <i>V. Joukov</i>

*2*

## APPLICABLE RULES AND STATUTES

### 37 CFR 1.56. DUTY OF DISCLOSURE: INFORMATION MATERIAL TO PATENTABILITY (Applicable Portion)

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is canceled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is canceled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
- (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentability defines, to make sure that any material information contained therein is disclosed to the Office.

Information relating to the following factual situations enumerated in 35 USC 102 and 103 may be considered material under 37 CFR 1.56(a).

### 35 U.S.C. 102. CONDITIONS FOR PATENTABILITY: NOVELTY AND LOSS OF RIGHT TO PATENT

A person shall be entitled to a patent unless --

- (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for patent, or
- (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of the application for patent in the United States, or
- (c) he has abandoned the invention, or
- (d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or
- (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraph (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or
- (f) he did not himself invent the subject matter sought to be patented, or
- (g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other.

### 35 U.S.C. 103. CONDITIONS FOR PATENTABILITY; NON-OBVIOUS SUBJECT MATTER

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negative by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

### 35 U.S.C. 112. SPECIFICATION (Applicable Portion)

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

## DECLARATION OF PATENT APPLICATION AND POWER OF ATTORNEY

Atty. Docket No: 28113/32863  
OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled "RECEPTOR LIGAND," the specification of which (check one): ☐ is attached hereto; ☒ was filed on August 1, 1995 as Application Serial No. 08/510,133 and was amended on \_\_\_\_\_ (if applicable); ☐ was filed as PCT International Application No. \_\_\_\_\_ on \_\_\_\_\_ and was amended under Article 19 on \_\_\_\_\_ (if applicable). I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Priority Claimed

(Application Serial Number)	(Country)	(Day/Month/Year Filed)
_____	_____	_____
_____	_____	_____

Yes	No
<input type="checkbox"/>	<input type="checkbox"/>
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<input type="checkbox"/>	<input type="checkbox"/>

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below:

(Application Serial Number)	(Day/Month/Year Filed)
_____	_____
_____	_____

I hereby claim the benefit under 35 U.S.C. §120 of any United States application(s) or PCT international application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in 37 C.F.R. §1.56 which occurs between the filing date of the prior application(s) and the national or PCT international filing date of this application:

(Application Serial Number)	(Day/Month/Year Filed)	(Status: Patented, Pending or Abandoned)
_____	_____	_____
_____	_____	_____

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements are the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Alvin D. Shulman (19,412)	Trevor B. Joike (25,542)	Richard A. Schmitt (39,890)	James J. Napoli (32,161)
Donald J. Brott (19,490)	Timothy J. Vezeau (26,348)	Anthony Nimmo (30,920)	Richard M. LaBerge (32,254)
Owen J. Murray (22,111)	Carl E. Moore, Jr. (26,487)	Christine A. Dudrick (31,245)	Jeffrey W. Smith (31,455)
Allen H. Gerstein (22,218)	Richard H. Anderson (26,526)	Kevin D. Hogg (31,839)	Douglas C. Hochstetler (33,710)
Nate F. Scarpelli (22,320)	Patrick D. Ertel (26,877)	Jeffrey S. Sharp (31,879)	Cynthia L. Schaller (34,245)
Edward M. O'Toole (22,477)	James P. Zeller (28,491)	Donald J. Pochopie (32,167)	Robert M. Gerstein (34,824)
Michael F. Borun (25,447)	William E. McCracken (30,195)	Martin J. Hirsch (32,237)	

Send correspondence to: Thomas C. Meyers

FIRM NAME	PHONE NO.	STREET	CITY & STATE	ZIP CODE
Marshall, O'Toole, Gerstein, Murray & Borun	312-474-6300	6300 Sears Tower 233 South Wacker Drive	Chicago, Illinois	60606-6402

Full Name of First or Sole Inventor	Citizenship
Kari Alitalo	Finland
Residence Address - Street	Post Office Address - Street
Nyyrikintie 4A	Same
City (Zip)	City (Zip)
02100 Espoo	Same
State or Country	State or Country
FINLAND	Same
Date	Signature
November 30, 1995	[Signature]

See second page for additional inventor(s)

See reverse for relevant rules &amp; statutes

7. **Deposit Account and Refund Authorization**

The Commissioner is hereby authorized to charge any deficiency in the amount enclosed or any additional fees which may be required during the pendency of this application under 37 CFR 1.16 or 37 CFR 1.17 or under other applicable rules (except payment of issue fees), to Deposit Account No. 13-2855. A copy of this Transmittal is enclosed.

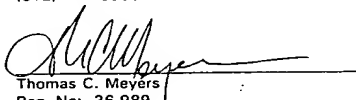
Please refund any overpayment to Marshall, O'Toole, Gerstein, Murray & Borun at the address below.

Please direct all future communications to Thomas C. Meyers, at the address below.

Respectfully submitted,

MARSHALL, O'TOOLE, GERSTEIN,  
MURRAY & BORUN  
6300 Sears Tower  
233 South Wacker Drive  
Chicago, Illinois 60606-6402  
(312) 474-6300

By:

  
Thomas C. Meyers  
Reg. No: 36,989

August 1, 1995

5. Filing Fee Calculation (37 CFR 1.16)

A. ☒ Utility Application

CLAIMS AS FILED - INCLUDING PRELIMINARY AMENDMENT (IF ANY)						
			SMALL ENTITY		OTHER THAN A SMALL ENTITY	
	NO. FILED	NO. EXTRA	RATE	FEE	RATE	FEE
BASIC FEE				\$365.00		\$730.00
TOTAL	12 - 20	= 0	X 11 =	\$	X 22 =	\$
INDEP.	3 - 3	= 0	X 38 =	\$	X 76 =	\$
<input type="checkbox"/> First Presentation of Multiple Dependent Claim			+ 120 =	\$	+ 240 =	
Filing Fee:				\$	OR	\$730.00

B. ☐ Design Application (\$150.00/\$300.00)

Filing Fee: \$ \_\_\_\_\_

C. ☐ Plant Application (\$245.00/\$490.00)

Filing Fee: \$ \_\_\_\_\_

D. Other Fees

☐ Recording Assignment (Fee -- \$40.00 per assignment) \$ \_\_\_\_\_

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached (Fee -- \$130.00) \$ \_\_\_\_\_

☐ Other \$ \_\_\_\_\_

Total Fees Enclosed **\$730.00**

6. Method of Payment of Fees

☒ Check in the amount of: **\$730.00**

☐ Charge Deposit Account No. 13-2855 in the amount of: \$ \_\_\_\_\_  
A copy of this Transmittal is enclosed.

☐ Not enclosed

3. Declaration or Oath

- ☐ Enclosed
  - ☐ Executed by (check all applicable boxes)
    - ☐ Inventor(s)
    - ☐ Legal representative of inventor(s)  
(37 CFR 1.42 or 1.43)
    - ☐ Joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached
      - ☐ The petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 are enclosed. See Item 5D below for fee.
- ☒ Not enclosed - the undersigned attorney or agent is authorized to file this application on behalf of the applicant(s). An executed declaration will follow.

4. Additional Papers Enclosed

- ☐ Preliminary Amendment
- ☐ Information Disclosure Statement
- ☐ Declaration of Biological Deposit
- ☒ Computer readable copy of sequence listing containing nucleotide and/or amino acid sequence
- ☐ Verified statement(s) claiming small entity status under 37 CFR 1.9 and 1.27
- ☐ Associate Power of Attorney
- ☐ Verified translation of a non-English patent application
- ☐ An assignment of the invention
- ☐ Certified copy(ies) of application(s):

COUNTRY	APPLICATION NO.	FILED

from which priority under 35 USC 119 is claimed ☐ is(are) attached.  
☐ will follow.

☐ Other





08/510133

**PATENT APPLICATION**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Docket No: 28113/32863

**PATENT APPLICATION TRANSMITTAL**

**Box Patent Application**  
**Assistant Commissioner for Patents**  
**Washington, D. C. 20231**

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Kari Alitalo and Vladomir Joukov

Title: "Receptor Ligand"

**1. Type of Application**

This new application is for a

☒ utility patent.


☐ design patent.

**2. Application Papers Enclosed**

- |                                     |  |
|-------------------------------------|--|
| 1                                   | Title Page   |
| 40                                  | Pages of Specification (excluding Claims, Abstract & Drawings) |
| 2                                   | Pages of Claims  |
| 1                                   | Page of Abstract   |
| 12                                  | Sheets of Drawings (Figs. 1 to 12)                             |
| <input type="checkbox"/>            | Formal   |
| <input checked="" type="checkbox"/> | Informal   |

**CERTIFICATION UNDER 37 CFR 1.10**

I hereby certify that this Patent Application Transmittal and the documents referred to as enclosed therewith are being deposited with the United States Postal Service on August 1, 1995, in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 utilizing the "Express Mail Post Office to Addressee" service of the United States Postal Service under Mailing Label No. EG473138672US.

  
Thomas C. Meyers

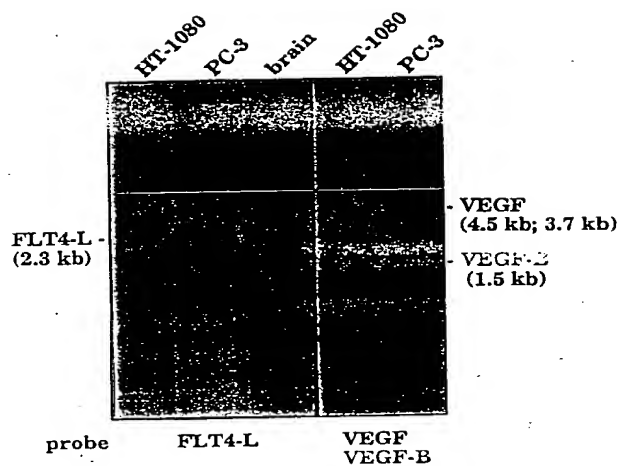


FIGURE 12

08/510131

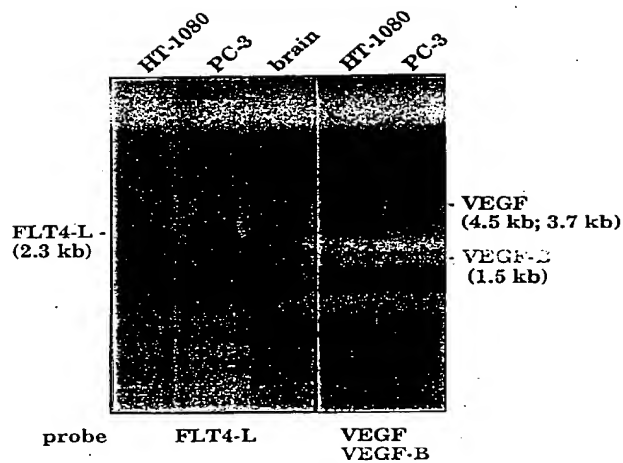


FIGURE 12

08/510133

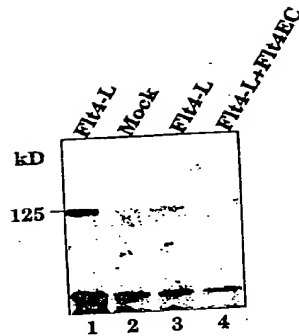


FIGURE 11

08/51013

50  
 PDGF-A 1 .MRTWACLLL LGCGYLAHAL AEEAEIPREL IERLARSQIH SIRDQLRLL  
 PDGF-B MNRCWA.LFL SLCCYLRLVS AEGDPIPEEL YEMLSDSHSIR SFDDLQRLH  
 PLGF .....MP VMRLFPCLQ LLAGLAL...  
 VEGF .....MNFLLSVWH WSLALLLYLH  
 FLT4-L .....MTVLYPEYWK MYKQLRKGG

100  
 PDGF-A 51 IDSVGAEDAL ETSLEAHGSH AINHVPKRP VPIRRKRSI.....EEAIP  
 PDGF-B GDP.GEEDGA ELDLNMTRSH SGGELES...LARGRRSLG SLTIAEPAMI  
 PLGF PAVPPQWAL SA.....GNGSSEVEVV P.FQEVWG...R  
 VEGF HAKWSQAAPH AE.....GGGQNHHEVV K.FMDVYQ...R  
 FLT4-L WQHNREQANL NSRTEETIKF AAAYHNTAIL KSIDNEW...K

150  
 PDGF-A 101 AVCKTRTVIY EIPRSQVDPT SANFLIMHPC VEVKRCIGCC NTSSVVCQPS  
 PDGF-B AECKTRTEVF EISRLIDRT NANFLVWHPC VEVORCSGCC MNRNVQCRPT  
 PLGF SYCRALERLV DVVSEY..PS EVEHMFSPSC VSLLRCTGCC GDENLHVVPV  
 VEGF SYCHPIETLV DIFQEY..PD EIEYIFKPS VPLMRCIGCC NDEGLECVPT  
 FLT4-L TCMPREVCI DVGKEF..GV ATNTFFKPC VSVYRCGCC NSEGLCMNT

200  
 PDGF-A 151 RVHHRSVKVA KVEYVRKKPK LKEVQVRLEE HLEAA.....AT.....  
 PDGF-B QVQLRFPVQR KIEIVRKKPI FKATVTLED HLAAC.....ETVAAARPVT  
 PLGF ETANVTMQLL KIRSG..DRP .SYVELTFSQ HVRLERPLR EKMKPERC..  
 VEGF EESNITMQIM RIKPH..QGQ .HIGEMSFLQ HNKLEPRPK DRARQENP..  
 FLT4-L STSYLSKTLF EITVPLSQGP .KPVITISFAN HTSRMSKL DVYRQVHSII

250  
 PDGF-A 201 ..SNLNPDRH EEETDVR... ..GKHRKFKHTH DKTALKETLG  
 PDGF-B RSPGGSQEQR AKTPQTRVTI RTVRVRPPK ..SDAGDDSTDG  
 PLGF .....GDAVPRR... ..KAROLELNER  
 VEGF .....CGPCSERRKH LRVQDPQTCCK CSCKNTDSRC  
 FLT4-L RRLSPATLPQ CQAANKTCPT NYMWNNHICR CLAQEDFMFS

300  
 PDGF-A 251 .....  
 PDGF-B A.....  
 PLGF .....  
 VEGF TCRCDKPRR.....  
 FLT4-L FHDICGFNKE LDEETCQCVC RAGLRPASCG PHKELDRNSC QCVCKNKLFP

350  
 PDGF-A 301 .....  
 PDGF-B .....  
 PLGF .....  
 VEGF .....  
 FLT4-L SQCGANREFD ENTQCQCVR TCPRNQPLNP GKACECTES PQKCLLKGGK

395  
 PDGF-A 351 .....  
 PDGF-B .....  
 PLGF .....  
 VEGF .....  
 FLT4-L FHHOTCSCYR RPCTNRQKAC EPGFSYSEEV CRCVPSYWKR PQMS

FIGURE 10

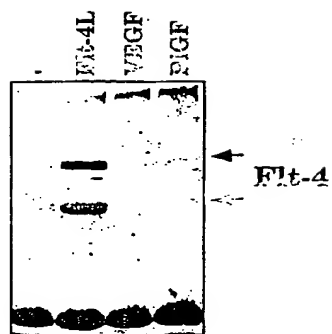
08/510139

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 10 30 50  
 TrpLysMetTyrLysCysGlnLeuArgLysGlyGlyTrpGlnHisAsnArgGluGlnAla  
 TCGAAAATGTACAAGTGTACGCTAAGGAAAGGAGGCTGGCAACATAACAGAGAACAGGCC  
 70 90 110  
 AsnLeuAsnSerArgThrGluGluThrIleLysPheAlaAlaAlaHisTyrAsnThrGlu  
 AACCTCAACTCAAGGACAGAGAGACTATAAAATTTGCTGCAGCACATTATAATACAGAG  
 130 150 170  
 IleLeuLysSerIleAspAsnGluTrpArgLysThrGlnCysMetProArgGluValCys  
 ATCTTGAAGATATTGATAATGAGTGGAGAAAGACTCAATGCATGCCACGGAGGTGTGT  
 190 210 230  
 IleAspValGlyLysGluPheGlyValAlaThrAsnThrPhePheLysProProCysVal  
 ATAGATCTCGGGAAGGAGTTTGGAGTCCGACAAACACCTTCTTTAAACCTCCATGTGTG  
 250 270 290  
 SerValTyrArgCysGlyGlyCysCysAsnSerGluGlyLeuGlnCysMetAsnThrSer  
 TCCGTCTACAGATCTGGCGCTTCTGCTCCCAATAGTACGGGGTGCAGTGCATGAACACAGC  
 310 330 350  
 ThrSerTyrLeuSerLysThrLeuPheGluIleThrValProLeuSerGlnGlyProLys  
 ACGAGCTACCTCAGCAAGACGTTATTGAAATTACAGTGCCTCTCTCAAGGCCCCAA  
 370 390 410  
 ProValThrIleSerPheAlaAsnHisThrSerCysArgCysMetSerLysLeuAspVal  
 CCAGTAACAATCAGTTTTCCTCAATCAGCTTCTGCGGATGCATGTCTAACTGGATGTT  
 430 450 470  
 TyrArgGlnValHisSerIleIleArgArgSerLeuProAlaThrLeuProGlnCysGln  
 TACAGACAAGTTTATTCATTATTAGACGTTCCCTGCCAGCAACACTACCAGATGTTCAG  
 490 510 530  
 AlaAlaAsnLysThrCysProThrAsnTyrMetTrpAsnAsnHisIleCysArgCysLeu  
 CGACGGAACAAGACCTGCCCCACCAATTACATGTGGAATAATCACATCTGCAGATGCTG  
 550 570 590  
 AlaGlnGluAspPheMetPheSerSerAspAlaGlyAspAspSerThrAspGlyPheHis  
 CCTCAGGAAGATTTTATGTTTCTCGGATGCTGGAGATGACTCAACAGATGCCATCCAT  
 610 630 650  
 AspIleCysGlyProAsnLysGluLeuAspGluGluThrCysGlnCysValCysArgAla  
 GACATCTGTGGACCAACAAGGAGCTGCATGAAGAGACCTGTCTAGTCTGCTGCAGAGCG  
 670 690 710  
 GlyLeuArgProAlaSerCysGlyProHisLysGluLeuAspArgAsnSerCysGlnCys  
 GGGCTTCGGCTGCCAGCTGTGGAACCCACAAAGAACTAGACAGAACTCATGCCAGTGT  
 730 750 770  
 ValCysLysAsnLysLeuPheProSerGlnCysGlyAlaAsnArgGluPheAspGluAsn  
 GTCTCTAAAACAACTCTTCCCGACCAATGTGGGGCAACCGAGAATTTGATGAAAAC  
 790 810 830  
 ThrCysGlnCysValCysLysArgThrCysProArgAsnGlnProLeuAsnProGlyLys  
 ACATGCCAGTGTGTATGTAAAGAACCCTCCCCAGAAATCAACCCCTAAATCCTGGAAAA  
 850 870 890  
 CysAlaCysGluCysThrGluSerProGlnLysCysLeuLeuLysGlyLysPheHis  
 TGTGCTGTGTAATGTACAGAAAGTCCACAGAAATGCTGTGTTAAAGGAAGAAGTTCCAC  
 910 930 950  
 HisGlnThrCysSerCysTyrArgArgProCysThrAsnArgGlnLysAlaCysGluPro  
 CACCAACATGCAGCTGTACAGAGCGGCATGTACGAACCGCCAGAACCTTGTGAGCCA  
 970 990 1010  
 GlyPheSerTyrSerGluGluValCysArgCysValProSerTyrTrpLysArgProGln  
 GGATTTTCATATAGTGAAGAACTGTCTCTCTCTCTCTCTCTATATTGGAAGAACACAA  
 1030 1050 1070  
 MetSerEnd  
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 1090 1110 1130

FIGURE 9

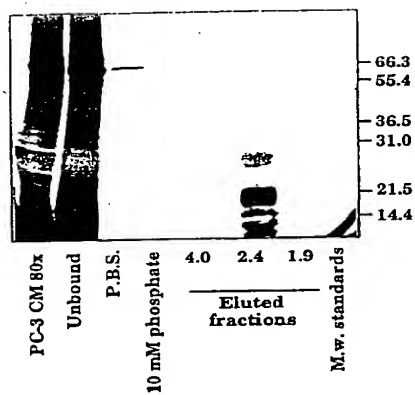
08/51013

FIGURE 8



08/510133

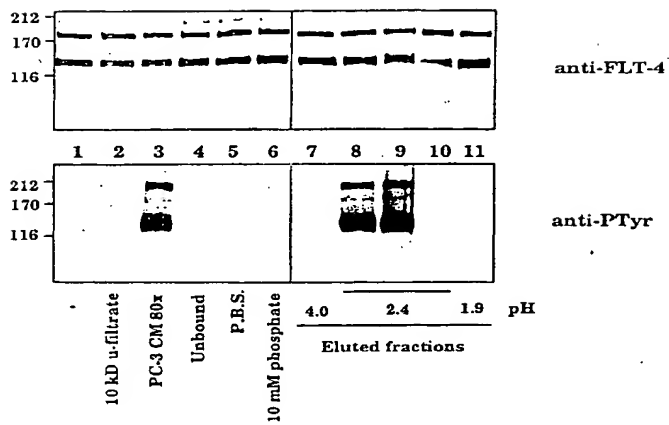
FIGURE 7





08/51013

FIGURE 6



08/51013

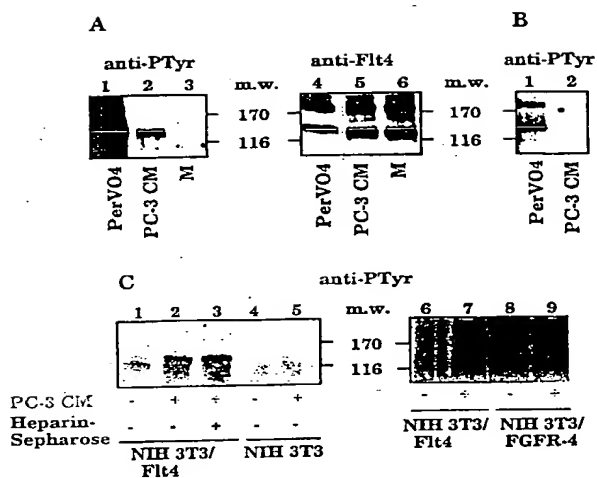


FIGURE 5

08/51015

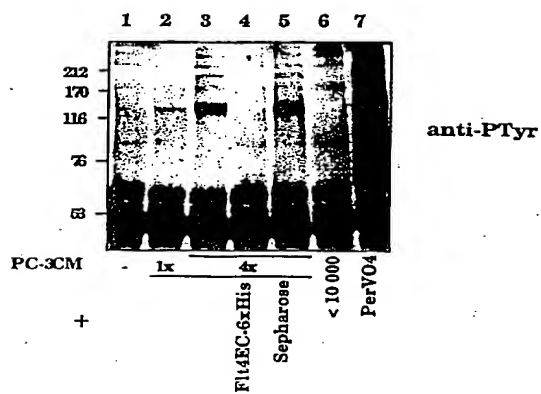
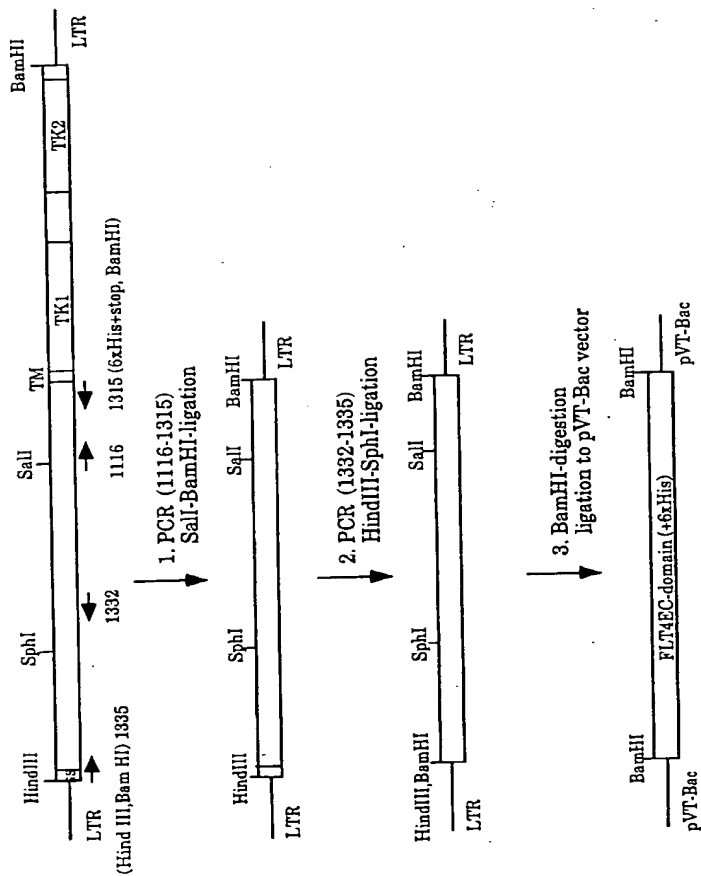


FIGURE 4

Figure 3



08/5101

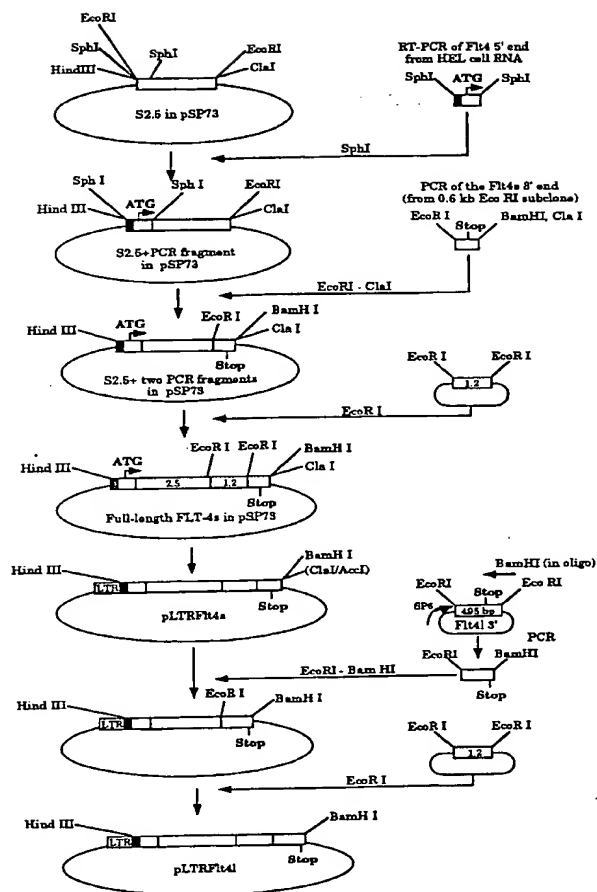
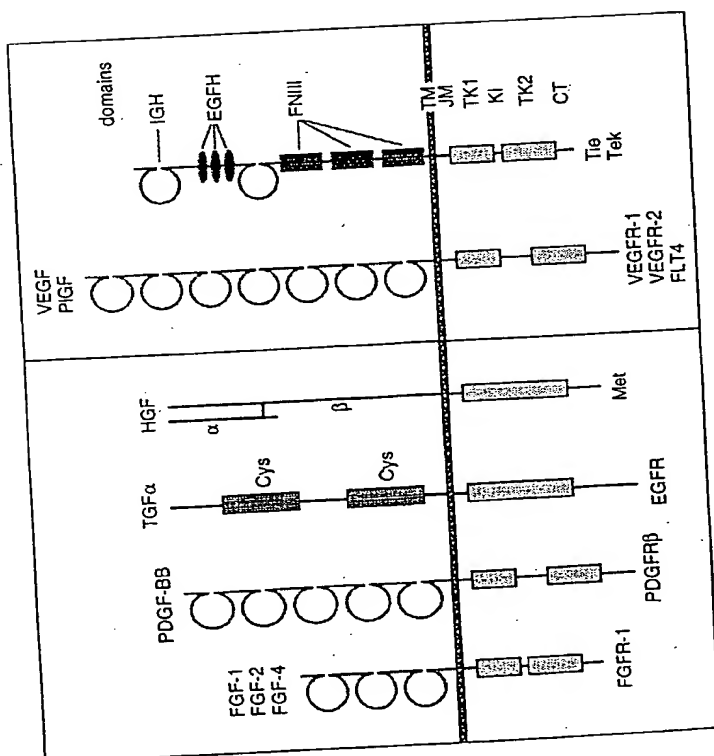


FIGURE 2

08/510133

FIGURE 1



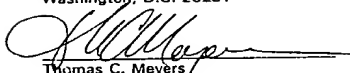


JOINT INVENTORS

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Date of Deposit: August 1, 1995

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Assistant Commissioner for Patents,  
Washington, D.C. 20231

  
Thomas C. Meyers

APPLICATION FOR  
UNITED STATES LETTERS PATENT

**SPECIFICATION**

---

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Kari Alitalo a citizen of Finland, residing at  
Nyyrikintie 4A, 02100 Espoo, Finland, and Vladimir Joukov a citizen of  
Finland, residing at Topeliuksenkatu 32G8, 00290 Helsinki, Finland, have  
invented a new and useful "RECEPTOR LIGAND", of which the following is a  
specification.



08/510133

- 43 -

#### ABSTRACT

Provided are ligands for the receptor tyrosine kinase, Flt4.  
Also provided are cDNAs and vectors encoding the ligand, pharmaceutical compositions and diagnostic reagents.



10. The fragment according to claim 8 comprising approximately the first 180 amino acids shown in SEQ ID NO: 32.

11. An antibody which is specifically reactive with the Flt4 ligand.

~~12. A pharmaceutical composition comprising a peptide according to claim 2 in a pharmaceutically-acceptable diluent, adjuvant, or carrier.~~

add  
C<sup>5</sup>

add  
D<sup>1</sup>

CLAIMS

1. A purified and isolated peptide which specifically binds to the Flt4 receptor tyrosine kinase.

2. A purified and isolated peptide having the amino acid sequence shown in SEQ ID NO: 33.

3. A nucleic acid encoding the purified and isolated peptide according to claim 2.

4. The nucleic acid according to claim 3 having the sequence shown in SEQ ID NO: 32.

5. A vector comprising the nucleic acid according to claim 4.

6. The vector according to claim 5, wherein said vector is plasmid pFlt4, deposited as ATCC accession No. .

7. A host cell comprising the vector according to claim 6.

8. A fragment of the purified and isolated peptide according to claim 2 which is capable of specifically binding to an Flt4 receptor tyrosine kinase.

9. The fragment according to claim 8 having an apparent molecular weight of 23 kD under reducing conditions.

[illegible]

AGC TAAGATTGTA CTGTTTTCCTA GTTCATCGAT TTTCTATTAT GGAAAACTGT 1658  
Ser  
GTTGCCACAG TAGAACTGTC TGTGAACAGA GAGACCCCTTG TGGGTCCATG CTAACAAAGA 1718  
CAAAAGTCTG TCTTTCCTGA ACCATGTGGA TAACTTTACA GAAATGGACT GGAGTCATC 1778  
TGCAAAAGGC CTCCTGTAAA GACTGGTTTT CTGCCAATGA CCAACAGCC AAGATTTTCC 1838  
TCTCTGATT TCTTTAAAG AATGACTATA TAATTTATTT CCACTAAAAA TATTGTTCT 1898  
GCATTCATTT TTATAGCAAC AACAATTGGT AAAACTCACT GTGATCAATA TTTTATATC 1958  
ATGCAAAATA TGTTTAAAT AAAATGAAAA TTGTATTAT 1997

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 419 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala  
1 5 10 15  
Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe  
20 25 30  
Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala  
35 40 45  
Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser  
50 55 60  
Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met  
65 70 75 80  
Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln  
85 90 95  
Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala  
100 105 110  
His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys  
115 120 125  
Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe  
130 135 140  
Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr  
145 150 155 160  
Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr  
165 170 175  
Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Gln Ile Thr Val Pro Leu  
180 185 190

GGG GGT TGC TGC AAT AGT GAG GGG CTG CAG TGC ATG AAC ACC AGC ACG Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr 165 170 175	885
AGC TAC CTC AGC AAG ACG TTA TTT GAA ATT ACA GTG CCT CTC TCT CAA Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln 180 185 190	933
GGC CCC AAA CCA GTA ACA ATC AGT TTT GCC AAT CAC ACT TCC TGC CGA Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg 195 200 205 210	981
TGC ATG TCT AAA CTG GAT GTT TAC AGA CAA GTT CAT TCC ATT ATT AGA Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg 215 220 225	1029
CGT TCC CTC CCA GCA ACA CTA CCA CAG TGT CAG GCA GCG AAC AAG ACC Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Lys Thr 230 235 240	1077
TGC CCC ACC AAT TAC ATG TGG AAT AAT CAC ATC TGC AGA TGC CTG GCT Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala 245 250 255	1125
CAG GAA GAT TTT ATG TTT TCC TCG GAT GCT GGA GAT GAC TCA ACA GAT Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp 260 265 270	1173
GGA TTC CAT GAC ATC TGT GGA CCA AAC AAG GAG CTG GAT GAA GAG ACC Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr 275 280 285 290	1221
TGT CAG TGT GTC TGC AGA GCG GGG CTT CGG CCT GCC AGC TGT GGA CCC Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro 295 300 305	1269
CAC AAA GAA CTA GAC AGA AAC TCA TGC CAG TGT GTC TGT AAA AAC AAA His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys 310 315 320	1317
CTC TTC CCC AGC CAA TGT GGG GCC AAC CGA GAA TTT GAT GAA AAC ACA Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr 325 330 335	1365
TGC CAG TGT CTA TGT AAA AGA ACC TGC CCC AGA AAT CAA CCC CTA AAT Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn 340 345 350	1413
CCT GGA AAA TGT GCC TGT GAA TGT ACA GAA AGT CCA CAG AAA TGC TTG Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu 355 360 365 370	1461
TTA AAA GGA AAG AAG TTC CAC CAC CAA ACA TGC AGC TGT TAC AGA CGG Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg 375 380 385	1509
CCA TGT ACG AAC CGC CAG AAG GCT TGT GAG CCA GGA TTT TCA TAT AGT Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser 390 395 400	1557
GAA CAA GGA GGT GGT GGT GGT GGT TTA TAT TAT AAA ACA CAA CAA ATT Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Tyr Lys Arg Pro Gln Met 405 410 415	1605

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 352..1608

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCCCCCCCC (TCTCCAAAA AGCTACACCG ACGCGGACCG CGGCGGCGTC CTCCTCGCC	60
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TTTTACCTGA CACCCGCCGC CTTTCCCCGG CACTGGCTGG GAGGGCGCCC TGCAAAGTTG	180
GGAACGCGGA GCCCGGACC CGTCCCGCC GCCTCCGGCT CGCCGAGGG GGGTCGCCGG	240
GAGGAGCCCC GGGGAGAGGG ACCAGGAGGG GCCCGCGGCC TCGCAGGGGC GCCCGCGCCC	300
CCAACCTGC (CCCGCCAGC GGACCGGTCC CCCACCCCCG CTCCTTCCAC C ATG CAC	357
Met His	
1	
TTG CTG GGC TTC TTC TCT GTG GCG TGT TCT CTG CTC GCC GCT GCG CTG	405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu	
5 10 15	
CTC CCG GGT CCT CGC GAG GCG CCC GCC GCC GCG GCC TTC GAG TCC	453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe Glu Ser	
20 25 30	
GGA CTC GAC CTC TCG GAC GCG GAG CCC GAC GCG GGC GAG GCC ACG GCT	501
Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala Thr Ala	
35 40 45 50	
TAT GCA AGC AAA GAT CTG GAG GAG CAG TTA CGG TCT GTG TCC AGT GTA	549
Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser Val	
55 60 65	
GAT GAA CTC ATG ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AAG	597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys	
70 75 80	
TGT CAG CTA AGG AAA GGA GGC TGC CAA CAT AAC AGA GAA CAG GCC AAC	645
Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn	
85 90 95	
CTC AAC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT	693
Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala His Tyr	
100 105 110	
AAT ACA GAG ATC TTG AAA AGT ATT GAT AAT GAG TGG AGA AAG ACT CAA	741
Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln	
115 120 125 130	
TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTC	789
Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val	
135 140 145	
GCG ACA AAC ACC TTC TTT AAA CCT CCA TGT GTG TCC GTC TAC AGA TGT	837
Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys	
150 155 160	

- 40 -

```

Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys Gly Gly Cys
      85                               90                               95

Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu
      100                               105                               110

Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu Ser Gln Gly Pro Lys
      115                               120                               125

Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser Cys Arg Cys Met Ser
      130                               135                               140

Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile Ile Arg Arg Ser Leu
      145                               150                               155                               160

Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr
      165                               170                               175

Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys Leu Ala Gln Glu Asp
      180                               185                               190

Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser Thr Asp Gly Phe His
      195                               200                               205

Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys
      210                               215                               220

Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro His Lys Glu
      225                               230                               235                               240

Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys Asn Lys Leu Phe Pro
      245                               250                               255

Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys
      260                               265                               270

Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys
      275                               280                               285

Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly
      290                               295                               300

Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr
      305                               310                               315                               320

Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val
      325                               330                               335

Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro Gln Met Ser
      340                               345                               350

```

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1997 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

AAG GAG CTG GAT GAA GAG ACC TGT CAG TGT GTC TGC AGA GCG GGG CTT	726
Lys Glu Leu Asp Glu Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu	
215 220 225	
CGG CCT GCC AGC TGT GGA CCC CAC AAA GAA CTA GAC AGA AAC TCA TGC	774
Arg Pro Ala Ser Cys Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys	
235 240 245	
CAG TGT GTC TGT AAA AAC AAA CTC TTC CCC AGC CAA TGT GGG GCC AAC	822
Gln Cys Val Cys Lys Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn	
250 255 260	
CGA GAA TTT GAT GAA AAC ACA TGC CAG TGT GTA TGT AAA AGA ACC TGC	870
Arg Glu Phe Asp Glu Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys	
265 270 275	
CCC AGA AAT CAA CCC CTA AAT CCT GGA AAA TGT GCC TGT GAA TGT ACA	918
Pro Arg Asn Gln Pro Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr	
280 285 290	
GAA AGT CCA CAG AAA TGC TTG TTA AAA GGA AAG AAG TTC CAC CAC CAA	966
Glu Ser Pro Gln Lys Cys Leu Leu Lys Gly Lys Lys Phe His His Gln	
295 300 305 310	
ACA TGC AGC TGT TAC AGA CGG CCA TGT ACG AAC CGC CAG AAG GCT TGT	1014
Thr Cys Ser Cys Tyr Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys	
315 320 325	
GAG CCA GGA TTT TCA TAT AGT GAA GAA GTG TGT CGT TGT GTC CCT TCA	1062
Glu Pro Gly Phe Ser Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser	
330 335 340	
TAT TGG AAA AGA CCA CAA ATG AGC TAAGATTGTA CTGTTTCCA GTTCATCGAT	1116
Tyr Trp Lys Arg Pro Gln Met Ser	
345 350	
TTTCTATTAT CGAAACTGT GTTG	1140

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 350 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu	
1 5 10 15	
Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln Ala Asn Leu Asn Ser	
20 25 30	
Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala His Tyr Asn Thr Glu	
35 40 45	
Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys Thr Gln Cys Met Pro	
50 55 60	
Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe Gly Val Ala Thr Asn	
65 70 75 80	



(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 37..1089

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GAGCAGTTAC GGTCTGTGTC CAGTGTAGAT GAATC	ATG ACT GTA CTC TAC CCA	54
	Met Thr Val Leu Tyr Pro	
	1 5	
GAA TAT TGG AAA ATG TAC AAG TGT CAG CTA AGG AAA GGA GGC TGG CAA	102	
Glu Tyr Trp Lys Met Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln		
10 15 20		
CAT AAC AGA GAA CAG GCC AAC CTC AAC TCA AGG ACA GAA GAG ACT ATA	150	
His Asn Arg Glu Gln Ala Asn Leu Asn Ser Arg Thr Glu Thr Ile		
25 30 35		
AAA TTT GCT GCA GCA CAT TAT AAT ACA GAG ATC TTG AAA AGT ATT GAT	198	
Lys Phe Ala Ala Ala His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp		
40 45 50		
AAT GAG TGG AGA AAG ACT CAA TGC ATG CCA CGG GAG GTG TGT ATA GAT	246	
Asn Glu Trp Arg Lys Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp		
55 60 65 70		
GTG GGG AAG GAG TTT GGA GTC GCG ACA AAC ACC TTC TTT AAA CCT CCA	294	
Val Gly Lys Glu Phe Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro		
75 80 85		
TGT GTG TCC GTC TAC AGA TGT GCG GGT TGC TGC AAT AGT GAG GGG CTG	342	
Cys Val Ser Val Tyr Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu		
90 95 100		
CAG TGC ATG AAC ACC AGC ACG AGC TAC CTC AGC AAG ACG TTA TTT GAA	390	
Gln Cys Met Asn Thr Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu		
105 110 115		
ATT ACA GTG CCT CTC TCT CAA GGC CCC AAA CCA GTA ACA ATC AGT TTT	438	
Ile Thr Val Pro Leu Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe		
120 125 130		
GCC AAT CAC ACT TCC TGC CGA TGC ATG TCT AAA CTG GAT GTT TAC AGA	486	
Ala Asn His Thr Ser Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg		
135 140 145 150		
CAA GTT CAT TCC ATT ATT AGA CGT TCC CTG CCA GCA ACA CTA CCA CAG	534	
Gln Val His Ser Ile Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln		
155 160 165		
TGT CAG GCA GCG AAC AAG ACC TGC CCC ACC AAT TAC ATG TGG AAT AAT	582	
Cys Gln Ala Ala Asn Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn		
170 175 180		
CAC ATC TGC AGA TGC CTG GCT CAG GAA GAT TTT ATG TTT TCC TCG GAT	630	
His Ile Cys Arg Cys Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp		
185 190 195		
GCT GCA GAT GAC TCA ACA GAT GCA TTC CAT CAC ATC TGT GGA CCA AAC	678	
Ala Gly Asp Asp Ser Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn		
200 205 210		

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 25 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AAGAGACTAT AAAATTCGCT GCAGC

25

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

CCCTCTAGAT GCATGCTCGA

20

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 1140 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCACTATAGG GAGACCCAAG C

21

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 219 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TCACTATAGG GAGACCCAAG CTTGGTACCG AGCTCGGATC CACTAGTAAC GGCCGCCAGT  
 GTGGTGGAAAT TCGACGAACT CATGACTGTA CTCTACCCAG AATATTGGAA AATGTACAAG  
 TGTACAGCTAA GGCAAGGAGG CTGGCAACAT AACAGAGAAC AGGCCAACCT CAACTCAAGG  
 ACAGAAGAGA CTATAAAATT CGCTGCAGCA CACTACAAC

60

120

180

219

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

ACAGAGAACA GGCCAACC

18

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TCTAGCATTT AGGTGACAC

19

(2) INFORMATION FOR SEQ ID NO:28:

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TCAGTGTGTGT AGTGTGCTG

19

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala His Tyr Asn Thr Glu  
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TAATACGACT CACTATAGGG

20

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTTGTAGTGT GCTGCAGCGA ATTT

24

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Phe Ala Ala Ala His Tyr Asn  
1 5

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Glu Thr Ile Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAYTTNARD ATYTCNGT

18

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 5 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Thr Glu Ile Leu Lys  
1 5

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATTCGCTGCA GCACACTACA AC

22

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

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(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGCTTG GATCCAAGTG GCTACTCCAT GACC

34

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGCGCTGTG ATGTGCACCA

20

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Glu Glu Thr Ile Lys Phe Ala Ala His Tyr Asn Thr Glu Ile  
1 5 10 15  
Leu Lys

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 17 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTATATGATAA CTATATAA

17

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:

24

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCATCGATGG ATCCCGATGC TGCTTAGTAG CTGT

34

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro	Met	Thr	Pro	Thr	Thr	Tyr	Lys	Gly	Ser	Val	Asp	Asn	Gln	Thr	Asp
1				5					10					15	
Ser	Gly	Met	Val	Leu	Ala	Ser	Glu	Glu	Phe	Glu	Gln	Ile	Glu	Ser	Arg
		20					25					30			
His	Arg	Gln	Glu	Ser	Gly	Phe	Arg								
	35					40									

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CTGGAGTCGA CTGGCGGAC T

21

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 60 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCGGATCCC TAGTGATGGT GATGGTGATG TCTACCTTCG ATCATGCTGC CCTTATCCTC

60

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG 60  
GACTCCTGGA 70

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACATGCATGC CCGCCCGTGC ATCC 24

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGAATTCCT CATGACCCCA AC 22

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT 33

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATTTAGGTGA CACTATA 17



- 30 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Alitalo, Kari  
Joukov, Vladomir
- (ii) TITLE OF INVENTION: Receptor Ligand
- (iii) NUMBER OF SEQUENCES: 35
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
  - (B) STREET: 6300 Sears Tower, 233 South Wacker Drive
  - (C) CITY: Chicago
  - (D) STATE: Illinois
  - (E) COUNTRY: United States of America
  - (F) ZIP: 60606-6402
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: 08/510,133
  - (B) FILING DATE: 08/01/95
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Gass, David A.
  - (B) REGISTRATION NUMBER: 38,153
  - (C) REFERENCE/DOCKET NUMBER: 28113/32863
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 312/474-6300
  - (B) TELEFAX: 312/474-0448
  - (C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGTCCTCGCT GTCCTGTCT

20

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 70 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

5 solution, 100 mg/ml salmon sperm DNA and  $1 \times 10^6$  cpm of the labelled probe/ml. The blot was washed at room temperature for 2 x 30 minutes in 2 x SSC containing 0.05% SDS and then for 2 x 20 min at <sup>52°C</sup> ~~52°C~~ in 0.1 x SSC containing 0.1% SDS. The blot was then exposed at <sup>70°C</sup> ~~-70°C~~ for three days using intensifying screens and Kodak XAR film. Both cell lines expressed an Flt4 ligand mRNA of about 2.3 kb, as well as VEGF and VEGF-B mRNA:s (Fig. 12).

Growth Factors 8, 245-252, 1993) one determines that the region critical for receptor activation by the Flt4 ligand is contained within its first approximately 180 amino acid residues.

On the other hand, the difference between the molecular  
5 weights of the purified ligand and the open reading frame of the Flt4 precursor clone may be due to the fact that the soluble ligand was produced from an alternatively spliced mRNA which would also be present in the PC-3 cells, from which the isolated ligand was derived. To isolate such alternative cDNA clones one uses cDNA fragments of the deposited clone  
10 and PCR primers made according to the sequence provided as well as techniques standard in the art to isolate or amplify alternative cDNAs from the PC-3 cell cDNA library. One may also amplify using reverse transcription (RT)-PCR directly from the PC-3 mRNA using the primers provided in the sequence of the Flt4-L clone. Alternative cDNAs can be  
15 sequenced from the resulting cDNA clones. One can also isolate genomic clones corresponding to the Flt4-L transcript from a human genomic DNA library using methods standard in the art and to sequence such clones or their subcloned fragments to reveal the corresponding exons. Alternative exons can then be identified by a number of methods standard in the art,  
20 such as heteroduplex analysis of cDNA and genomic DNA and they can subsequently be characterized.

#### EXAMPLE 12

##### Expression of the Flt4-L gene

25 Expression of transcripts corresponding to the Flt4 ligand was analysed by hybridization of Northern blots containing isolated polyA<sup>+</sup> RNA from HT-1080 and PC-3 human tumor cell lines. The probe was the radioactively labelled insert of the 2.1 kb cDNA clone (specific activity  $10^8$ - $10^9$  cpm/mg of DNA). The blot was hybridized overnight at 42°C, using 50% formamide, 5 x SSPE buffer, 2% SDS, 10 x Denhardt's

may be involved, for example, in the regulation of secretion, solubility, stability, cell surface localization or activity of the Flt4 ligand. Interestingly, at least one cysteine motif of the BRP3 type is also found in the VEGF carboxy terminal amino acid sequences.

5           Thus, the Flt4 mRNA may be first translated into a precursor from the mRNA corresponding to the Flt4-L clone, from which the mature ligand is derived by proteolytic cleavage. To define the mature Flt4 ligand product one first expresses the cDNA clone, which is deposited in the pcDNA1 expression vector, in cells, such as COS cells and use  
10           antibodies generated against Flt4-L-encoded peptides, such as amino terminal 23 amino acid peptide or bacterial Flt4 fusion proteins, such as a GST-fusion protein, to raise antibodies against the VEGF-homologous domain of Flt4 ligand. One then follows the biosynthesis and processing of the Flt4 ligand in the transfected cells by pulse-chase analysis using  
15           radioactive cysteine for labelling of the cells, immunoprecipitation and gel electrophoresis. Using antibodies against the two domains of the product of the Flt4-L clone material for radioactive or nonradioactive aminoterminal sequence analysis is isolated. The determination of the NH<sub>2</sub>-terminal  
20           sequence of the carboxyl terminal fragment allows for identification of the proteolytic processing site. This is confirmed by site-directed mutagenesis of the amino acid residues adjacent to the cleavage site, which would prevent the cleavage.

On the other hand, the Flt4 ligand is characterized by progressive 3' deletions in the 3' coding sequences of the Flt4 ligand  
25           precursor clone, resulting in <sup>in vitro</sup> C-~~COOH~~-terminal truncations of its protein product. The activities of such truncated forms are assayed by, for example, studying Flt4 autophosphorylation induced by the truncated  
30           proteins when applied to cultures of cells, such <sup>as</sup> NIH3T3 cells expressing LTRFlt4. By extrapolation from studies of the structure of the related platelet derived growth factor (PDGF, reference Heldin et al.,

87-  
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receptor (lane 2). When the concentrated conditioned medium was preabsorbed with 20  $\mu$ l of a slur of Flt4EC domain coupled to Sepharose (see example 4), no phosphorylation was obtained (lane 4), showing that the activity responsible for Flt4 autophosphorylation was indeed the Flt4 ligand. Thus, these results demonstrate that the Flt4-L plasmid vector clone having an approximately 2.1 kb insert and containing the open reading frame shown in Fig. 9 is expressed into a Flt4 ligand in cells transfected with the Flt4-L expression vector clone, and thus is biologically active. The sequence encoded by that open reading frame is shown in SEQ ID NO: 33. Plasmid ~~FLT4-L~~ has been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852 as accession number ~~72231~~ <sup>72231</sup>.

However, the predicted molecular weight of the mature protein product deduced from this reading frame is 35,724 and the Flt4 ligand from PC-3 cell cultures had an approximate molecular weight of 23 kD under reducing conditions. It is thus possible that the Flt4 mRNA may be first translated into a precursor, from which the mature ligand is derived by proteolytic cleavage. The difference in the observed molecular weight of the isolated Flt4 ligand and the deduced molecular weight of the disclosed open reading frame of the Flt4 ligand sequence may then derive from sequences in the carboxyl terminal region of the latter. Also, the Flt4 ligand may be glycosylated at two putative N-linked glycosylation sites conforming to the consensus which can be identified in the deduced Flt4 ligand amino acid sequence (N-residues <sup>underlined</sup> marked in Fig. 10).

The carboxyl terminal amino acid sequences, which increase the predicted molecular weight of the Flt4 ligand subunit in comparison with other ligands of this family show a pattern of spacing of cysteine residues reminiscent of the Balbiani ring protein 3 (BRP3) sequence (Dignam and Case, Gene 88, 133-140, 1990). Such a sequence may encode an independently folded domain present in a Flt4 ligand precursor and it

in that figure, after the putative signal sequence the open reading frame terminates in a TAA stop codon 317 amino acid residues further downstream from the signal sequence. When compared with sequences in the GenBank Database, the predicted protein product of this reading frame was found to be homologous with the predicted amino acid sequences of the PDGF/VEGF family of growth factors, as shown in Figure-10.

#### EXAMPLE 11

##### Stimulation of Flt4 autophosphorylation by the protein product of the Flt4 ligand vector

The 2.1 kb insert of the Flt4-L clone in pcDNA1 vector containing the open reading frame encoding the sequence shown in Fig-9 (SEQ ID NO: 32) was cut out from the vector using *HindIII* and *NotI* restriction enzymes, isolated from a preparative agarose gel and ligated to the corresponding sites in the pREP7 expression vector (Invitrogen). The pREP7 vector containing the above cloned insert was transfected into 293-EBNA cells (Invitrogen) using the calcium phosphate transfection method (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). About 48 hours after transfection the medium of the transfected cells was changed to DMEM medium lacking fetal calf serum and incubated for 36 h. The thus conditioned medium was then collected, centrifuged at 5000xg for 20 minutes, the supernatant was used concentrated 5-fold using Centrprep 10 (Amicon) and to stimulate NIH3T3 cells expressing LTRFlt4l, as in Example 4. The cells were lysed, immunoprecipitated using anti-Flt4 antiserum and analysed by Western blotting using anti-phosphotyrosine antibodies.

As can be seen from Fig. 11, lanes 1 and 3, the conditioned medium from two different dishes of the transfected cells stimulated Flt4 autophosphorylation in comparison with the medium from mock-transfected cells, which gave only background levels of phosphorylation of the Flt4

31) (sense-primer corresponding to nucleotides 2179-2199 of the pcDNA1 vector). The amplified product was subjected to digestion with *EcoRI* (Boehringer Mannheim) to remove the portion of the DNA sequence amplified from the pcDNA1 vector and the resulting 153 bp fragment encoding the 5' end of the Flt4 ligand was labeled with [<sup>32</sup>P]-dCTP using the Klenow fragment of *E. coli* DNA polymerase I (Boehringer Mannheim). That fragment was used as a probe for hybridization screening of the amplified PC-3 cell cDNA library.

Filter replicas of the library were hybridized with the radioactively labeled probe at 42 °C for 20 hours in a solution containing 50% formamide, 5xSSPE, 5x Denhardt's solution, 0.1% SDS and 0.1 mg/ml denatured salmon sperm DNA. Filters were washed twice in 4xSSC, 0.1% SDS for 30 minutes at room temperature, then twice for 30 minutes at 65 °C and exposed overnight.

On the basis of autoradiography, 10 positive recombinant bacterial colonies hybridizing with the probe were chosen from the library. Plasmid DNA was purified from these colonies and analysed by *EcoRI* and *NorI* digestion and agarose gel electrophoresis followed by ethidium bromide staining. The ten plasmid clones were divided into three groups on the basis of the presence of insert sizes of approximately 1.7, 1.9 and 2.1 kb, respectively. Inserts of plasmids from each group were sequenced using the T7 oligonucleotide as a primer and walking primers for subsequent sequencing reactions.

Sequence analysis showed that all clones contain the open reading frame encoding the NH2-terminal sequence of the Flt4 ligand. Furthermore, the 2.1 and 1.9 kb clones also contained sequences encoding the signal sequence. The 5' end of the 1.7 kb clone began within the signal sequence-encoding portion. Dideoxy sequencing was continued using walking primers in the downstream direction. An 1140 nucleotide portion of the sequence of the longest clone is shown in Figure 9. As can be seen

#### EXAMPLE 9

##### Amplification of the 3'-end of cDNA encoding the Flt4 ligand

Based upon the amplified 5'-sequence of the clones encoding the Flt4 ligand, two pairs of non-overlapping nested primers were designed to amplify the 3'-portion of the clones. The sense-strand primer 5'-ACAGAGAACAGGCCAACC-3' (SEQ ID NO: 26) and antisense-strand primer 5'-TCTAGCATTTAGGTGACAC-3' (SEQ ID NO: 27) corresponding to nucleotides 2311-2329 of the pcDNA1 vector were used in a first "touchdown" PCR. The annealing temperature of the reaction was decreased 1°C every two cycles from 72°C to 52°C, at which temperature 15 additional cycles were carried out. The annealing time was 1 minute and extension at each cycle was carried out at 72°C for 3 minutes. DNA fragments of several sizes were obtained in the first amplification. Those products were diluted 1:200 in water and reamplified in PCR using the second pair of primers: 5'-AAGAGACTATAAAATTCGCTGCAGC-3' (SEQ ID NO: 28) and 5'-CCCTCTAGATGCATGCTCGA-3' (SEQ ID NO: 29) (antisense-strand primer corresponding to nucleotides 2279-2298 of the pcDNA1 vector). Two DNA fragments were obtained, having sizes of 1350 bp and 570 bp. Those fragments were cloned into a pCRII vector and the inserts of the clones were sequenced. Both of these fragments were found to contain sequences encoding an amino acid sequence homologous to the VEGF sequence.

#### EXAMPLE 10

##### Screening the PC-3 cell cDNA library using the 5' PCR fragment of Flt4 ligand cDNA

A 219 bp 5'-terminal fragment of Flt4 ligand cDNA was amplified by PCR using the 5' PCR fragment described above and primers 5'-GTTGTAGTGTGCTGCAGCGAATTT-3' (antisense-strand primer, SEQ ID NO: 30) and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO:



minute. Multiple amplified DNA fragments were obtained in the first reaction. The products of the first amplification (1 ul of a 1:100 dilution in water) were used in the second amplification reaction employing the nested primers 5'-GTTGTAGTGTGCTGCAGCGAATT-3' (SEQ ID NO: 22), an antisense-strand primer corresponding to amino acid residues 6-13 (KFAAAHYN, SEQ ID NO: 23) of the Flt4 ligand and 5'-TCACTATAGGGAGACCCAAGC-3' (SEQ ID NO: 24), a sense-strand primer corresponding to nucleotides 2179-2199 of the pcDNAI vector. The sequences of these sense and antisense primers overlapped with the 3' ends of the corresponding primers used in the first PCR. "Touchdown" PCR was carried out by decreasing the annealing temperature from 72 °C to 66 °C and continuing with 18 additional cycles at 66 °C. The annealing time was 1 minute and extension at each cycle was carried out at 72 °C for 2 minutes. One major product of about 220 bp and three minor products of about 270 bp, 150 bp, and 100 bp were obtained.

The amplified fragment of approximately 220 bp was cut out from the agarose gel, cloned into a pCRII vector using the TA cloning kit (Invitrogen) and sequenced. Three recombinant clones were analysed and they contained the sequence 5'-

TCACTATAGGGAGACCCAAGCTTGGTACCGAGCTCGGATCCACTA  
GTAACGGCCGCCAGTGTGGTGAATTGACGGAAGCTCATGACTGTA  
CTCTACCCAGAATATTGGAAAATGTACAAGTGTCAAGCTAAGGCCAA  
GGAGGCTGGCAACATAACAGAGAACAGGCCAACCTCAACTCAAG  
GACAGAAGAGACTATAAAATTGCTGCAGCACACTACAAC 3'

(SEQ ID NO: 25). The beginning of the sequence represents the pcDNAI vector and the underlined sequence represents the amplified product of the 5'-end of the insert. The ATG codon located upstream of that sequence in the same reading frame is followed by an open reading frame containing the amplified product of the putative signal sequence and the first 13 amino acid residues of the secreted Flt4 ligand.

42°C for 1 minute. The amplified fragment was cloned into a pCR II vector (Invitrogen) using the TA cloning kit (Invitrogen) and sequenced using the radioactive dideoxynucleotide sequencing method of Sanger. Six clones were analysed and all contained the sequence encoding the expected peptide  
5 (amino acids 2-18 of the Flt4 ligand precursor). Nucleotide sequence spanning the region from the third nucleotide of codon 6 to the third nucleotide of codon 13 (the extension region) was identical in all six clones: 5'-ATTCGCTGCAGCACACTACAAC-3' (SEQ ID NO: 18) and  
10 thus was considered to represent an amplified product from the unique sequence encoding part of the amino terminus of the Flt4 ligand.

#### EXAMPLE 8

##### Amplification of the 5'-end of the cDNA encoding the Flt4 ligand

Based on the unique nucleotide sequence encoding the N-  
15 terminus of the isolated Flt4 ligand, two pairs of nested primers were design to amplify in two subsequent PCR-reactions the complete 5'-end of the corresponding cDNAs from 1 µg of DNA from the above-described PC-3 cDNA library. First, amplification was performed with primer 5'-TCNGTGTGTAGTGTGCTG-3' (SEQ ID NO: 19) which is the antisense-  
20 strand primer corresponding to amino acid residues 9-15 (AAHYNTE, SEQ ID NO: 20) and sense-strand primer 5'-TAATACGACTCACTATAGGG-3' (SEQ ID NO: 21) corresponding to the T7 RNA promoter of the pcDNA1 vector used for construction of the library. "Touchdown" PCR was used as disclosed in Don, *et al.*, *Nucl. Acids Res.*, 19: 4008 (1991), incorporated  
25 by reference herein. The annealing temperature of the two first cycles was 62 °C and subsequently the annealing temperature was decreased in every other cycle by 1 °C until a final temperature of 53 °C was reached, at which temperature 16 additional cycles were carried out. Annealing time was 1 minute and extension at each cycle was conducted at 72 °C for 1

according to the instructions included in the kit. The library was estimated to contain about  $10^6$  independent recombinants with an average insert size of approximately 1.8 kb.

#### EXAMPLE 7

##### 5 Amplification of the unique nucleotide sequence encoding the Flt4 ligand

Degenerate oligonucleotides were designed based on the N-terminal amino acid sequence of the isolated Flt4 ligand and were used as primers in a polymerase chain reaction (PCR) to amplify cDNA encoding the Flt4 ligand from a PC-3 cell library.

10 The PCR was carried out using  $1 \mu\text{g}$  of DNA from the amplified PC-3 cDNA library and a mixture of sense-strand primers comprising 5'-GCAGARGARACNATHAA-3' (SEQ ID NO: 14) (wherein R is A or G, N is A, G, C or T and H is A, C or T), encoding amino acid residues 2-6 (EETIK, SEQ ID NO: 15) and antisense-strand primers 5'-GCAYTTNARDATYTCNGT-3' (SEQ ID NO: 16) (wherein Y is C or T and D is A, G or T), corresponding to amino acid residues 14-18 (TEILK, SEQ ID NO: 17). Three extra nucleotides (GCA) were added to the 5'-terminus of each primer to increase annealing stability. Two successive  
20 PCR runs were carried out using 1U per reaction of DynaZyme, a thermostable DNA polymerase (F-500L, Finnzymes), in a buffer supplied by the manufacturer (10 mM Tris-HCl, pH 8.8 at 25 °C, 1.5 mM  $\text{MgCl}_2$ , 50 mM KCl, 0.1% Triton-X100) at an extension temperature of 72 °C. The first PCR run was carried out for 43 cycles. The first three cycles  
25 were run at annealing temperature 33 °C for 2 minutes and the remaining cycles were run at 42 °C for 1 minute.

The region of the gel containing a weak band of the expected size (57 bp) was cut out from the gel and eluted. The eluted material was reamplified for 30 cycles using the same primer pairs described above at

detected in the fractions containing Flt4 stimulating activity (corresponding to lanes 8 and 9). That polypeptide was not found in the other chromatographic fractions. On the other hand, all other components detected in the two active fractions were also distributed in the starting material and in small amounts in the other washing and elution steps after their concentration. Similar results were obtained in three independent affinity purifications, indicating that the 23 kD polypeptide specifically binds to Flt4 and induces its tyrosine phosphorylation.

Fractions containing the 23 kD polypeptide were combined, dried in a SpeedVac concentrator and subjected to SDS-PAGE in a 12.5 % gel. The proteins from the gel were then electroblotted to Immobilon-P (PVDF) transfer membrane (Millipore, Malborough, MA) and visualized by staining of the blot with Coomassie blue R-250. The region containing only the stained 23 kD band was cut from the blot and was subjected to N-terminal amino acid sequence analysis in a Prosite Protein Sequencing System (Applied Biosystems, Foster City, CA). The data were analyzed using a 610A Data Analysis System (Applied Biosystems). Analysis revealed a single N-terminal sequence of NH<sub>2</sub>-XEETIKFAAAHYNTEILK-COOH (SEQ ID NO: 13).

#### EXAMPLE 6

##### Construction of PC-3 cell cDNA library in a eukaryotic expression vector.

Poly-A + RNA was isolated from five 15 cm diameter confluent dishes of PC-3 cells by a single step method using oligo(dT) (Type III, Collaborative Research) cellulose affinity chromatography (Sambrook et al., Molecular Cloning, A Laboratory Manual; Cold Spring Harbor Laboratory Press, 1989). The yield was 70 µg. Six µg of the poly-A + RNA was used to prepare an oligo(dT)-primed cDNA library in the mammalian expression vector pcDNA I and the Librarian kit of Invitrogen

rolling tube at room temperature for 3 hours. All subsequent purification steps were at +4°C. The affinity matrix was then transferred to a column (Pharmacia) with an inner diameter of 15 mm and washed successively with 100 ml of PBS and 50 ml of 10 mM Na-phosphate buffer (pH 6.8).  
5 Bound material was eluted step-wise with 100 mM glycine-HCl, successive 6 ml elutions having pHs of 4.0, 2.4, and 1.9. Several 2 ml fractions of the eluate were collected in tubes containing 0.5 ml 1 M Na-phosphate (pH 8.0). Fractions were mixed immediately and dialysed in 1 mM Tris-HCl (pH 7.5). Aliquots of 75 µl each were analyzed for their ability to  
10 stimulate tyrosine phosphorylation of Flt4. The ultrafiltrate, 100 µl aliquots of the concentrated conditioned medium before and after ligand affinity chromatography, as well as 15-fold concentrated fractions of material released from the Flt4 extracellular domain-Sepharose matrix during the washings were also analyzed for their ability to stimulate Flt4  
15 tyrosine phosphorylation.

As shown in Figure 6, lane 3, the concentrated conditioned medium induced prominent tyrosine phosphorylation of Flt4 in transfected NIH3T3 cells overexpressing Flt4. This activity was not observed in conditioned medium taken after medium was exposed to the Flt4  
20 Sepharose affinity matrix described above (lane 4). The specifically-bound Flt4-stimulating material was retained on the affinity matrix upon washes in PBS, 10 mM Na-phosphate buffer (pH 6.8), and at pH 4.0 (lanes 5-7, respectively), and it was eluted in the first two 2 ml aliquots at pH 2.4 (lanes 8 and 9). A further decrease of the pH of the elution buffer did not  
25 cause release of additional Flt4-stimulating material (lane 11).

Small aliquots of the chromatographic fractions were concentrated in a SpeedVac concentrator (Savant, Farmingdale, N.Y.) and subjected to SDS-PAGE under reducing conditions with subsequent silver staining of the gel. As shown in Figure 7, the major polypeptide, having a  
30 molecular weight of approximately 23 kD (reducing conditions), was

medium with 50  $\mu$ l of the Flt4 extracellular domain coupled to CNBr-activated sepharose CL-4B (Pharmacia; about 1mg of Flt4 EC domain/ml sepharose resin) completely abolished Flt4 tyrosine phosphorylation. Similar pretreatment of the conditioned medium with unsubstituted  
5 sepharose CL-4B did not affect stimulatory activity, as shown in Figure 4, lane 5. Also, the flow through obtained after concentration, which contained proteins of less than 10,000 molecular weight, did not stimulate Flt4 phosphorylation, as shown in Figure 4, lane 6.

The foregoing data show that PC-3 cells produce a ligand  
10 which binds to the extracellular domain of Flt4 and activates this receptor.

#### EXAMPLE 5

##### Purification of the Flt4 Ligand

The ligand expressed by PC-3 cells as characterized in  
Example 3 was purified and isolated using a recombinant<sup>ly</sup>-produced Flt4  
15 extracellular domain in affinity chromatography.

Two harvests of serum-free conditioned medium, comprising a total of 8 L were collected from 500 confluent 15 cm diameter culture dishes containing confluent layers of PC-3 cells. The conditioned medium was clarified by centrifugation at 10,000 x g and concentrated 80-fold using  
20 an Ultrasette Tangential Flow Device (Filtron, Northborough, MA) with a 10 kD cutoff Omega Ultrafiltration membrane according to the manufacturer's instruction. Recombinant Flt4 extracellular domain was expressed in a recombinant baculovirus cell system and purified by affinity chromatography on Ni-agarose (Ni-NTA affinity column obtained from  
25 Qiagen). The purified extracellular domain was coupled to CNBr-activated Sepharose CL-4B at a concentration of 5 mg/ml and used as an affinity matrix for ligand affinity chromatography.

Concentrated conditioned medium was incubated with 2 ml of the recombinant Flt4 extracellular domain-Sepharose affinity matrix in a

mM Tris, pH7.5 before analysis in SDS-PAGE. Polypeptides were transferred to nitrocellulose and analyzed by Western blotting using Flt4- or phosphotyrosine-specific antisera and the ECL method (Amersham International, Buckinghamshire, England). Anti-phosphotyrosine monoclonal antibodies (anti-PTyr; PY20) were purchased from Transduction Laboratories (Lexington, Kentucky). In some cases, the filters were retained with a second antibody after stripping. The stripping of the filters was done for 30 minutes at 50°C in 100 mM 2-mercaptoethanol, 2% SDS, 62.5 mM Tris-HCl pH 6.7 with occasional agitation.

As shown in Figure 4, the PC-3 cell conditioned medium stimulated tyrosine phosphorylation of a 125 kD polypeptide when Flt4 expressing NIH3T3 cells were treated with the indicated preparations of media, lysed, and the lysates were immunoprecipitated with anti-Flt4 antiserum followed by SDS-PAGE, Western blotting, and staining using anti-PTyr antibodies. The resulting band was weakly phosphorylated upon stimulation with unconcentrated PC-3 conditioned medium (lane 2). The 125 kD band comigrated with the tyrosine phosphorylated, processed form of the mature Flt4 from pervanadate-treated cells (compare lanes 2 and 7 of Fig. 4, see also Figure 5A). Comigration was confirmed upon restaining with anti-Flt4 antibodies as is also shown in Figure 5A (panel on the right). In order to show that the 125 kD polypeptide is not a non-specific component of the conditioned medium reactive with anti-phosphotyrosine antibodies, 15  $\mu$ l of conditioned medium was separated by SDS-PAGE, blotted on nitrocellulose and the blot was stained with anti-PTyr antibodies. No signal was obtained (Fig. 5B). Also, unconditioned medium failed to stimulate Flt4 phosphorylation, as shown in Figure 4, lane 1.

As shown in Figure 4, lane 3, stimulating activity was considerably increased when the PC-3 conditioned medium was concentrated four-fold using a Centricon-10 concentrator (Amicon). Figure 4, lane 4, shows that pretreatment of the concentrated PC-3 conditioned

domain.

#### EXAMPLE 4

##### Isolation of Flt4 Ligand from Conditioned Media

An Flt4 ligand according to the invention was isolated from  
5 conditioned media from PC-3 prostatic adenocarcinoma cell line CRL1435  
from the American Type Culture Collection and cultured as instructed by  
the supplier in Ham's F-12 Nutrient mixture (GIBCO) containing 7% fetal  
calf serum. In order to prepare the conditioned media, confluent PC-3 cells  
10 were cultured for 7 days in Ham's F-12 Nutrient mixture (GIBCO) in the  
absence of fetal bovine serum. Medium was then cleared by centrifugation  
at 10,000 g for 20 minutes. The medium was then screened to determine  
its ability to induce tyrosine phosphorylation of Flt4 by exposure to  
NIH3T3 cells which had been transfected with Flt4-encoding cDNA using  
the pLTRFlt4l vector. For receptor stimulation experiments, subconfluent  
15 NIH3T3 cells were starved overnight in serum-free DMEM medium  
(GIBCO) containing 0.2% BSA. The cells were stimulated with the  
conditioned media for 5 minutes, washed twice with cold PBS containing  
100 uM vanadate and lysed in RIPA buffer (10 mM Tris pH 7.5, 50 mM  
NaCl, 0.5% sodium deoxycholate, 0.5% Nonidet P40 (BDH, Poole,  
20 England), 0.1% SDS, 0.1 U/ml Aprotinin (Boehringer Mannheim), 100  
uM vanadate) for receptor immunoprecipitation analysis. The lysates were  
centrifuged for 20 minutes at 15,000 x g. The supernatants were incubated  
for 2 hours on ice with 3 ul of the antiserum against the Flt4 C-terminus  
described in Example 2 and also in Pajusola, *et al. Oncogene* 8: 2931-  
25 2937, (1993), incorporated by reference herein.

After a 2 hour incubation in the presence of anti-Flt4  
antiserum, protein A-Sepharose (Pharmacia) was added and incubation was  
continued for 45 minutes with rotation. The immunoprecipitates were  
washed three times with the immunoprecipitation buffer and twice with 10



column (Qiagen, Hilden, Germany) followed by a stop codon, and an added Bam HI site. The amplified fragment was digested with *Sall* and *BamHI* and used to replace a unique *Sall-BamHI* fragment in the LTRFlt4 vector shown in Figure 3. The *Sall-BamHI* fragment that was replaced encodes the Flt4 transmembrane and cytoplasmic domains.

The 5' end without the Flt4 signal sequence encoding region was amplified by PCR using the primer 1335 5'-

CCCAAGCTTGGATCCAAGTGGCTACTCCATGACC-3' (SEQ ID NO: 11) (the primer contains added *HindIII* (AAGCTT) and *BamHI* (GGATCC) restriction sites, which are underlined) and primer 1332 5'-

GTGCCTGTGATGTGCACCA-3' (SEQ ID NO: 12). The amplified fragment was digested with *HindIII* and *SphI* (the *HindIII* site (AAGCTT) is underlined in primer 1335 and the *SphI* site is within the amplified region of the Flt4 cDNA). The resulting *HindIII-SphI* fragment was used

to replace a *HindIII-SphI* fragment in the modified LTRFlt4 vector described immediately above (the *HindIII* site is in the 5' junction of the Flt4 insert with the pLTRpoly portion of the vector, the *SphI* site is in Flt4 cDNA). The resulting Flt4EC insert was then ligated as a *BamHI* fragment into the *BamHI* site in the pVTBac plasmid as disclosed in Tessier *et al.*,

*Gene* 98: 177-183 (1991), incorporated by reference herein. The orientation was confirmed to be correct by partial sequencing so that the open reading frame of the signal sequence-encoding portion of the vector continued in frame with the Flt4 sequence. That construct was transfected together with the baculovirus genomic DNA into SF-9 cells by lipofection.

Recombinant virus was purified, amplified and used for infection of High-Five cells (Invitrogen, San Diego, CA) using methods standard in the art. The Flt4 extracellular domain was purified from the culture medium of the infected High-Five cells using Ni-NTA affinity chromatography according to manufacturer's instructions (Qiagen) for binding and elution of the 6xHis tag encoded in the COOH-terminus of the recombinant Flt4 extracellular

chromatography using a glutathione-Sepharose 4B column. The purified protein was lyophilized, dissolved in phosphate buffered saline (PBS), mixed with Freund's adjuvant and used for immunization of rabbits at biweekly intervals using methods standard in the art (Harlow and Lane, 5 Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988). Antisera were used after the fourth booster immunization for immunoprecipitation of Flt4 from the transfected cells and clones expressing Flt4 were used for ligand stimulation analysis.

### EXAMPLE 3

#### 10 Construction of a Flt4 EC baculovirus vector and expression and purification of its product

The construction of an Flt4 extracellular domain (EC) baculovirus vector is schematically shown in Figure 3. The Flt4-encoding cDNA has been prepared in both a long form and a short form, each being 15 incorporated in a vector under control of the Moloney murine leukemia virus LTR promoter. The nucleotide sequence of the short form of the Flt4 receptor is available on the Genbank database as Accession No. X68203 and the specific 3' segment of the long form cDNA is available as Accession No. S66407.

20 The ends of a cDNA segment encoding Flt4 extracellular domain (EC) were modified as follows: The 3' end of Flt4 cDNA sequence (Genbank Accession Number <sup>X</sup>X68203) which encodes the extracellular domain was amplified using primer 1116 5'-  
CTGGAGTCGACTTGGCGGACT-3' (SEQ ID NO: 9, *Sall* site underlined)  
25 and primer 1315 5'-  
CGCGGATCCCTAGTGATGGTGATGGTGATGTCTACCTTCGATCATG  
CTGCCCTTAT CCTC-3' (SEQ ID NO: 10, *Bam*HI site underlined). The sequence complementary to that of primer 1315 continues after the Flt4 reading frame and encodes 6 histidine residues for binding to a Ni-NTA

and *Bam*HI and transferred in frame to LTRFlt4s vector fragment from which the coding sequences downstream of the *Eco*RI site at base pair 2535 (see sequence X68203) had been removed by *Eco*RI-*Bam*HI digestion. Again, the coding domain was completed by ligation of the 1.2 kb *Eco*RI fragment (base pairs 2535-3789 of sequence X68203) back into the resulting construct.

## EXAMPLE 2

### Production and analysis of Flt4 transfected cells

NIH3T3 cells (60 % confluent) were cotransfected with 5  $\mu$ g of the pLTRFlt4l construct and 0.25  $\mu$ g of the pSV2neo vector (ATCC) containing the neomycin phosphotransferase gene, using the DOTAP liposome-based transfection reagents (Boehringer Mannheim, Mannheim, Germany). One day after the transfection the cells were transferred into selection media containing 0.5 mg/ml geneticin (GIBCO, Grand Island, N.Y.). Colonies of geneticin-resistant cells were isolated and analysed for expression of the Flt4 proteins. Cells were lysed in boiling lysis buffer containing 3.3% SDS (sodium dodecyl sulphate), 125 mM Tris, pH 6.8. Protein concentrations of the samples were measured by the BCA method (Pierce, Rockford, IL). About 50  $\mu$ g protein of each lysate was analysed for the presence of Flt4 by 6% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using antisera against the carboxyl terminus of Flt4 and the ECL method (Amersham).

For production of anti-Flt4 antiserum the Flt4 cDNA fragment encoding the 40 carboxyterminal amino acid residues of the short form: NH<sub>2</sub>-PMTPTTYKG SVDNQTDSGM VLASEEFEQI ESRHRQESGFR-COOH (SEQ ID NO: 8) was cloned as a 657 bp *Eco*RI-fragment into the pGEX-III bacterial expression vector (Pharmacia) in frame with the glutathione-S-transferase coding region. The resulting GST-Flt4S fusion protein was produced in *E.coli* and purified by affinity

fragment, thus replacing unique *SphI* fragment of the S2.5 plasmid. The resulting vector was digested with *EcoRI* and *Clal* and ligated to a 138 bp PCR fragment amplified from the 0.6 kb *EcoRI* fragment (base pairs 3789 to 4416 in the Genbank X68203 sequence) which encodes the 3' end of

5 Flt4s shown in Figure 1 of Pajusola *et al.*, *Cancer Res.* 52:5738-5743, 1992, using the oligonucleotides 5'-CGGAATTCCC CATGACCCCA AC-3' (SEQ ID NO: 4) (forward, *EcoRI* site underlined) and 5'-CCATCGATGG ATCCTACCTG AAGCCGCTTT CTT-3' (SEQ ID NO: 5) (reverse, *Clal* site underlined). The coding domain was completed by

10 ligation of the 1.2 kb *EcoRI* fragment (base pairs 2535-3789 of sequence X68203) into the above construct. The complete cDNA was subcloned as a *HindIII-Clal*(blunted) fragment (this *Clal* site was also included in the 3' primer used to construct the 3' end of the coding sequence) to the pLTRpoly expression vector reported in Mäkelä *et al.*, *Gene*, 118: 293-294

15 (1992) (Genbank accession number X60280), incorporated by reference herein, using its *HindIII-Acc I*(blunted) restriction sites.

The long form of Flt4 was produced by replacing the 3'-end of the short form as follows: The 3' region of the Flt4l cDNA was PCR-amplified using a gene specific and a pGEM 3Z vector specific (SP6

20 promoter) oligonucleotide 5'-ATTTAGGTGACACTATA-3' (SEQ ID NO: 6) as reverse and forward primers, respectively, and an Flt4l cDNA clone containing a 495 bp *EcoRI* fragment extending downstream of the *EcoRI* site at nucleotide 3789 of the Genbank X68203 sequence (the sequence downstream of this *EcoRI* site is deposited as the Flt4 long form 3'

25 sequence having Genbank accession number S66407). The gene specific oligonucleotide contained a *BamHI* restriction site located right after the end of the coding region. The sequence of that (reverse primer) oligonucleotide was 5'-

30 CCATCGATGGATCCCGATGCTGCTTAGTAGCTGT-3' (SEQ ID NO: 7)(*BamHI* site is underlined). The PCR product was digested with *EcoRI*

HEL cells and double-stranded cDNA copy was synthesized using the Amersham cDNA Synthesis System Plus kit and a gene specific primer: 5'-TGTCCTCGCTGTCCTTGTCT-3' (SEQ ID NO: 1), which was located 195 bp downstream of the 5' end of clone S2.5. Double stranded cDNA was treated with T4 DNA polymerase to blunt the ends and cDNA was purified with Centricon 100 (Amicon Inc., Beverly, MA). Circularization was made in a total volume of 150 ul. The reaction mixture contained ligation buffer, 5% PEG-8000, 1 mM DTT and 8U of T4 DNA ligase (New England Biolabs). Ligation was carried out at 16°C for 16 hours. Fifteen µl of this reaction mix was used in a standard 100 ul PCR reaction containing 100 ng of specific primers including *SacI* and *PstI* restriction sites, present in this segment of the Flt4 cDNA, and 1 unit of Taq DNA polymerase (Perkin Elmer Cetus). Two rounds of PCR were performed using 33 cycles (denaturation at 95°C for 1 minute, annealing at 55°C for 2 minutes and elongation at 72°C for 4 minutes). The PCR mixture was treated sequentially with the *SacI* and *PstI* restriction enzymes and after purification with MagicPCR Preps (Promega) DNA fragments were subcloned into the pGEM3Zf(+) vector for sequencing. The sequence obtained corresponds to the 5' end of the Flt4s cDNA clone deposited in the Genbank Database as Accession No. X68203.

The sequence encoding the first 12 amino acid residues was added to the expression construct by ligating an *SphI* digested PCR fragment amplified using reverse transcription-PCR of polyA+ RNA isolated from the HEL cells using the oligonucleotides 5'-ACATGCATGC CACCATGCAG CGGGGCGCCG CGCTGTGCCT GCGACTGTGG CTCTGCCTGG GACTCCTGGA-3' (SEQ ID NO: 2)(forward primer, *SphI* site underlined, the translational start codon marked in bold follows an optimized Kozak consensus sequence Kozak, *Nucl. Acids Res.* 15: 8125-8148, 1987) and 5'-ACATGCATGC CCCGCCGT CATCC-3' (SEQ ID NO: 3) (reverse primer, *SphI* site underlined) to the 5' end of the S2.5

RNA of the ligand gene. Such a co-expressed region may be a function of the particular expression system used to obtain the ligand. The skilled artisan understands that in recombinant production of proteins, additional sequence may be expressed along with a functional peptide depending upon the particular recombinant construct used to express the protein, and subsequently removed to obtain the desired ligand. In some cases the recombinant ligand can be made lacking certain residues of the endogenous/natural ligand. Moreover, it is well-known in that conservative replacements may be made in a protein which do not alter the function of the protein. Accordingly, it is anticipated that such alterations are within the scope of the claims. It is intended that the precursor sequence shown in SEQ ID NO: 33 is capable of stimulating the Flt4 ligand without any further processing in a manner similar to that in which VEGF stimulates its receptor in its unprocessed form.

The following Examples illustrate preferred embodiments of the invention, wherein the isolation, characterization, and function of Flt4 ligands according to the invention is shown.

#### EXAMPLE 1

##### Production of pLTR-Flt4l expression vector

Construction of the LTR-Flt4l vector is schematically shown in Figure 2. The full-length Flt4s cDNA (Genbank Accession No. X68203) was assembled by first subcloning the S2.5 fragment, reported in Pajusola *et al.*, *Cancer Res.* 52:5738-5743 (1992), incorporated by reference herein, containing base pairs 56-2534 of the Flt4s into the *EcoRI* site of the pSP73 vector (Promega, Madison, WI).

Since cDNA libraries used for screening of Flt4 cDNAs did not contain its most 5' protein-coding sequences, inverse PCR was used for the amplification of the 5' end of Flt4 corresponding to the first 12 amino acid residues (MQRGAALCLRLW). PolyA+ RNA was isolated from the

Figure 7 shows results of gel electrophoresis of fractions from the Western analysis of Flt4 ligand isolated from PC-3 conditioned medium.

Figure 8 shows results of Western analysis of Flt4 autophosphorylation induced by either the Flt4 ligand, VEGF, or PIGF.

Figure 9 shows the nucleotide and deduced amino acid sequence of the coding portion of Flt4 ligand cDNA.

Figure 10 shows a comparison of the deduced amino acid sequences of PDGF-A, -B, two PIGF isoforms, four VEGF isoforms and Flt4 ligand.

Figure 11 shows the stimulation of autophosphorylation of the Flt4 receptor by conditioned medium from cells transfected with the Flt4-L expression vector

Figure 12 shows Northern blotting analysis of Flt4-L mRNA in tumor cell lines.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to novel growth factors which are ligands for the Flt4 receptor tyrosine kinase. Claimed ligands are members of a family of platelet-derived growth factors/vascular endothelial growth factors which promote mitosis and proliferation of vascular endothelial cells and/or mesodermal cells. Ligands recognizing the Flt4 receptor tyrosine kinase were purified from a PC-3 prostatic adenocarcinoma cell line (ATCC CRL1435). When applied to a population of cells expressing the Flt4 receptor, ligands of the invention stimulate autophosphorylation, resulting in receptor activation. The invention also provides inhibitors of the Flt4 receptor, including antibodies directed against the receptor. A ligand according to the invention may be coexpressed as a larger precursor which is cleaved to produce the ligand. A coexpressed region in some cases results from alternative splicing of

also be used to promote re-growth or permeability of lymphatic vessels in, for example, organ transplant patients. Ligands according to the invention may also be used to treat or prevent inflammation, edema, aplasia of the lymphatic vessels, lymphatic obstruction, elephantiasis, and Milroy's disease. Finally, Flt4 ligands may be used to stimulate lymphocyte production and maturation and to promote or inhibit trafficking of leukocytes between tissues and lymphatic vessels or to affect migration in and out of the thymus.

Inhibitors of the Flt4 ligand may be used to control endothelial cell proliferation and lymphangiomas. For example, such inhibitors may be used to arrest metastatic growth or spread or to control other aspects of endothelial cell expression and growth. Inhibitors include antibodies, antisense oligonucleotides, and peptides which block the Flt4 receptor.

#### DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing major endothelial cell receptor tyrosine kinases and growth factors involved in vasculogenesis and angiogenesis.

Figure 2 shows schematically the construction of the pLTRFlt4l expression vector

Figure 3 shows schematically the construction of the baculovirus vector encoding a secreted soluble Flt4EC domain

Figure 4 shows results of stimulation of Flt4 autophosphorylation by conditioned medium from PC-3 cell cultures.

Figure 5 shows that the tyrosyl phosphorylated polypeptide of Flt4-transfected cells stimulated with PC-3 conditioned medium is the 125 kD Flt4 polypeptide.

Figure 6 shows Western analysis of the Flt4 ligand activity isolated from PC-3 conditioned medium.



sequence shown in SEQ ID NO: 32.

The present invention also provides a cell line which produces an Flt4 ligand. The ligand may be purified and isolated directly from the cell culture medium. Also provided are vectors comprising DNA  
5 encoding the Flt4 ligand and host cells comprising the vectors. Vectors are capable of expressing the Flt4 ligand under the control of appropriate promoters and other control sequences.

Ligands according to the invention may be labeled with a detectable label and used to identify their corresponding receptors *in situ*.  
10 Antibodies, both monoclonal and polyclonal, may be made against a ligand of the invention according to standard techniques in the art. Such antibodies may be used in diagnostic applications to monitor angiogenesis, vascularization, lymphatic vessels and their disease states, wound healing, or certain hematopoietic or leukemia cells or they may be used to block or  
15 activate the Flt4 receptor. Labeled Flt4 ligand and anti-Flt4 ligand antibodies may be used as imaging agents in the detection of lymphatic vessels, high endothelial venules, and Flt4 receptors expressed in histochemical tissue sections. The ligand or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron  
20 dense, echogenic, or radioactive agent for imaging. Other, non-radioactive labels, such as biotin and avidin may also be used.

The present invention also provides diagnostic and clinical applications for claimed ligands. In a preferred embodiment, Flt4 ligands or precursors of the invention are used to accelerate angiogenesis *e.g.*  
25 during wound healing or to promote the endothelial functions of lymphatic vessels. Ligands may be applied in any suitable manner using an appropriate pharmaceutically-acceptable vehicle. Ligands may also be used to quantify future metastatic risk by assaying biopsy material for the presence of active receptors or ligands in a binding assay or kit using  
30 detectably-labeled ligand. An Flt4 ligand according to the invention may

observed in developing venous and presumptive lymphatic endothelia, but arterial endothelia appear negative. During later stages of development, Flt4 mRNA becomes restricted to developing lymphatic vessels. Only the lymphatic endothelia and some high endothelial venules express Flt4  
5 mRNA in adult human tissues and increased expression occurs in lymphatic sinuses in metastatic lymph nodes and in lymphangioma. These results support the theory of the venous origin of lymphatic vessels.

#### SUMMARY OF THE INVENTION

The present invention provides a ligand for the Flt4 receptor  
10 tyrosine kinase. In a preferred embodiment, the ligand comprises a fragment of the amino acid sequence shown in SEQ ID NO: 33 which specifically binds to the Flt4 receptor tyrosine kinase.

The present invention also provides a precursor of an Flt4 ligand, wherein the precursor comprises the amino acid sequence shown in  
15 SEQ ID NO: 33. The precursor is proteolytically cleaved upon expression to produce an approximately 23 kD peptide which is the Flt4 ligand. In a preferred embodiment of the invention, an Flt4 ligand is provided which is the cleavage product of the precursor peptide shown in SEQ ID NO: 33 and which has a molecular weight of approximately 23 kD under reducing  
20 conditions. The Flt4 ligand comprises approximately the first 180 amino acids shown in SEQ ID NO: 33.

Also in a preferred embodiment, nucleic acids encoding an Flt4 ligand precursor are presented. Due to the degeneracy of the genetic code, numerous such coding sequences are possible, each having in  
25 common the coding of the amino acid sequence shown in SEQ ID NO: 33, or portions thereof. Ligand precursors according to the invention, when expressed in an appropriate host cell, produce, via cleavage, a peptide which binds specifically to the Flt4 receptor tyrosine kinase. The nucleotide sequence encoding the Flt4 ligand is within the nucleotide


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1. Application as filed  
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11/26/91
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4/6/92

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# SEQUENCE ANALYSIS DEMONSTRATING VEGF-C AND VEGF-2 ARE THE SAME MOLECULE

VEGF-C	MHLLGFFSVACSLLAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL	60
VEGF-2	MHSLGFFSVACSLLAALLPGPREAPAAAAAFESGLDLSDAEPDAGEATAYASKDLEEQL	
*		
VEGF-C	RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK	120
VEGF-2	RSVSSVDELMTVLYPEYWKMYKCQLRKGGWQHNREQANLNSRTEETIKFAAAHYNTEILK	
VEGF-C	SIDNEWRTQCMPEVCI DVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY	180
VEGF-2	SIDNEWRTQCMPEVCI DVGKEFGVATNTFFKPPCVSVYRCGGCCNSEGLQCMNTSTSY	
VEGF-C	LSKTLFEITVPLSQGPKPVTISFANHNSCRMSKLDVYRQVHSIIRRSLPATLPQCQAAN	240
VEGF-2	LSKTLFEITVPLSQGPKPVTISFANHNSCRMSKLDVYRQVHSIIRRSLPATLPQCQAAN	
VEGF-C	KTCPTNYMWNNHICRLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR	300
VEGF-2	KTCPTNYMWNNHICRLAQEDFMFSSDAGDDSTDGFHDICGPNKELDEETCQCVCRAGLR	
VEGF-C	PASCGPHKELDRNSCQCVCNKLFPSQCGANREFDENTCQCVCCKRTCPRNQPLNPGKCAC	360
VEGF-2	PASCGPHKELDRNSCQCVCNKLFPSQCGANREFDENTCQCVCCKRTCPRNQPLNPGKCAC	
VEGF-C	ECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCPVPSYWKRPQMS	419
VEGF-2	ECTESPQKCLLKGKKFHHQTCSCYRRPCTNRQKACEPGFSYSEEVCRCPVPSYWRPQMS	

The consensus line:

- \* = Indicates substitutions that are neither conserved nor semi-conserved.
- : = indicates conserved substitutions.
- . = indicates semi-conserved substitutions.

AARONSON DECLARATION

APPENDIX I



114:521-523 (1992). On day two of quail development, the vascularized area of the yolk sac as well as the whole embryo show expression of VEGF. In addition, epithelial cells next to fenestrated endothelia in adult mice show persistent VEGF expression, suggesting a role in the maintenance of this specific endothelial phenotype and function.

Two high affinity receptors for VEGF have been characterized. These are VEGFR-1/Flt-1 (fms-like tyrosine kinase-1) and VEGFR-2/Kdr/Flk-1 (kinase insert domain containing receptor/fetal liver kinase-1). Those receptors are classified in the PDGF-receptor family, but they have seven rather than five immunoglobulin-like loops in their extracellular domain and they possess a longer kinase insert than normally observed in this family. The expression of VEGF receptors occurs mainly in vascular endothelial cells, although some may be present on monocytes and melanoma cells. Only endothelial cells have been reported to proliferate in response to VEGF and endothelial cells from different sources show different responses. Thus, the signals mediated through VEGFR-1 and VEGFR-2 appear to be cell type specific.

The Flt4 receptor tyrosine kinase is closely related in structure to the products of the VEGFR-1 and VEGFR-2 genes. Despite this similarity, the mature form of Flt4 differs from the VEGF receptors in that it is proteolytically cleaved in the extracellular domain into two disulfide-linked polypeptides. Pajusola *et al.*, *Cancer Res.*, 52:5738-5743 (1992). The 4.5 and 5.8 kb Flt-4 mRNAs encode polypeptides which differ in their C-termini due to the use of alternative 3' exons. The VEGFs do not show specific binding to Flt4 or induce its autophosphorylation.

Expression of Flt4 appears to be more restricted than expression of VEGFR-1 or VEGFR-2. The expression of Flt4 first becomes detectable by *in situ* hybridization in the angioblasts of head mesenchyme, the cardinal vein, and extraembryonically in the allantois of 8.5 day p.c. mouse embryos. In 12.5 day p.c. embryos the Flt-4 signal is

platelet derived growth factor, PDGF-BB, has been shown to be weakly angiogenic in the chick chorioallantoic membrane. Risau, *et al.*, *Growth Factors*, 7:261-266 (1992). Transforming growth factor  $\alpha$  (TGF $\alpha$ ) is an angiogenic factor secreted by several tumor cell types and by macrophages.

- 5 Hepatocyte growth factor (HGF), the ligand of the *c-met* proto-oncogene-encoded receptor, is also strongly angiogenic.

Recent evidence shows that there are endothelial cell specific growth factors and receptors that may be primarily responsible for the stimulation of endothelial cell growth, differentiation and certain  
10 differentiated functions. The best studied of these is vascular endothelial growth factor (VEGF), a member of the PDGF family. Vascular endothelial growth factor is a dimeric glycoprotein of disulfide-linked 23 kDa subunits, discovered because of its mitogenic activity toward endothelial cells and its ability to induce vessel permeability (hence its  
15 alternative name vascular permeability factor). Other reported effects of VEGF include the mobilization of intracellular calcium, the induction of plasminogen activator and plasminogen activator inhibitor-1 synthesis, stimulation of hexose transport in endothelial cells, and promotion of monocyte migration *in vitro*. Four VEGF isoforms encoded by distinct  
20 mRNA splice variants appear to be equally capable of stimulating mitogenesis in endothelial cells. However, each has a different affinity for cell surface proteoglycans, which behave as low affinity receptors for VEGF. The 121 and 165 amino acid isoforms of VEGF are secreted in a soluble form, whereas the isoforms of 189 and 206 amino acid residues  
25 remain cell surface associated and have a strong affinity for heparin.

The pattern of VEGF expression suggests its involvement in the development and maintenance of the normal vascular system and in tumor angiogenesis. During murine development, the entire 7.5 day post-coital (p.c.) endoderm expresses VEGF and the ventricular neuroectoderm  
30 produces VEGF at the capillary ingrowth stage Breier, *et al.*, *Development*,

migration, differentiation and survival. Dysfunction of the endothelial cell regulatory system is a key feature of many diseases. Most importantly, tumor growth and metastasis have been shown to be angiogenesis dependent. Folkman, *et al.*, *J. Biol. Chem.*, 267:10931-10934 (1992).

Key signals regulating cell growth and differentiation are mediated by polypeptide growth factors and their transmembrane receptors, many of which are tyrosine kinases. Autophosphorylated peptides within the tyrosine kinase insert and carboxyl-terminal sequences of activated receptors are commonly recognized by kinase substrates involved in signal transduction for the readjustment of gene expression in responding cells. Several families of receptor tyrosine kinases have been characterized. Van der Geer, *et al.*, *Ann. Rev. Cell Biol.*, 10:251-337 (1994). The major growth factors and receptors transducing angiogenic stimuli are schematically shown in Figure 1.

Fibroblast growth factors are also known to be involved in the regulation of angiogenesis. They have been shown to be mitogenic and chemotactic for cultured endothelial cells. Fibroblast growth factors also stimulate the production of proteases, such as collagenases and plasminogen activators, and induce tube formation by endothelial cells. Saksela, *et al.*, *Ann. Rev. Cell Biol.*, 4:93-126 (1988). There are two general classes of fibroblast growth factors, FGF-1 and FGF-2, both of which lack conventional signal peptides. Both types have an affinity for heparin and FGF-2 is bound to heparin sulfate proteoglycans in the subendothelial extracellular matrix from which it may be released after injury. Heparin potentiates the stimulation of endothelial cell proliferation by angiogenic FGFs, both by protecting against denaturation and degradation and dispersing the FGFs. Cultured endothelial cells express the FGF-1 receptor but no significant levels of other high-affinity fibroblast growth factor receptors.

Among other ligands for receptor tyrosine kinases, the



- 1 -

## RECEPTOR LIGAND

### FIELD OF THE INVENTION

The present invention generally relates to the field of genetic engineering and more particularly to growth factors for endothelial cells and growth factor genes.

### BACKGROUND OF THE INVENTION

Developmental growth, the remodelling and regeneration of adult tissues as well as solid tumor growth, can only occur when accompanied by blood vessel formation. Angioblasts and hematopoietic precursor cells differentiate from the mesoderm and form the blood islands of the yolk sac and the primary vascular system of the embryo. The development of blood vessels from these early (*in situ*) differentiating endothelial cells is termed vasculogenesis. Major embryonic blood vessels are believed to arise via vasculogenesis, whereas the formation of the rest of the vascular tree is thought to occur as a result of vascular sprouting from pre-existing vessels, a process called angiogenesis, Risau, *et al.*, *Devel. Biol.*, 125: 441-450 (1988).

Endothelial cells give rise to several types of functionally and morphologically distinct vessels. When organs differentiate and begin to perform their specific functions, the phenotypic heterogeneity of endothelial cells increases. Upon angiogenic stimulation, endothelial cells may re-enter the cell cycle, migrate, withdraw from the cell cycle and subsequently differentiate again to form new vessels that are functionally adapted to their tissue environment. Endothelial cells undergoing angiogenesis degrade the underlying basement membrane and migrate, forming capillary sprouts that project into the perivascular stroma. Ausprunk, *et al.*, *Microvasc. Rev.*, 14: 51-65 (1977). Angiogenesis during tissue development and regeneration depends on the tightly controlled processes of endothelial cell proliferation,

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S30	399	8/28	BKL
S14	2	1	1
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search update		4/10/00	CL
branch update		9/27/00	CL

INTERFERENCE SEARCHED			
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S30	399	9/27/00	CL
S14	2	↓	↓
	12		

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36. PTOZ 271  
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PATENT APPLICATION



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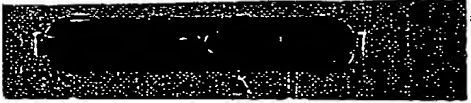
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2.	Statement 1-821	8-1-95
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7.	Restriction 30 Days	5-29-96
8.	Key 1	7-26-96
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12.	Jack Kistner	9-14-96
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16.	Letter 11/1/97	2-13-97
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18.	Discharge Affidavit	4/5/97
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20.	Timothy C. (OK)	6/11/97
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27.	Ass. Power of Attorney	6-22-00
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APPLICANTS  
KARI ALITALO, ESPOO, FINLAND; VLADIMIR JOUKOV, HELSINKI, FINLAND. *Slisk*

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RECEPTOR LIGAND  
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14. Amendment After Final Action  
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1/3/01

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**File History**  
**U.S. Patent No. 6,221,839**  
**Issued April 24, 2001**

1. Application as filed  
8/1/95
2. Information Disclosure Statement  
11/3/95
3. Notice to File Missing Parts  
11/20/95
4. Response to Notice to File Missing Parts  
12/19/95
5. Restriction Requirement  
5/29/96
6. Election with Traverse in Response to Restriction Requirement  
7/24/96
7. Preliminary Amendment  
8/12/96
8. Office Action  
9/10/96
9. Request for Amendment of Drawing  
2/10/97
10. Amendment and Reply Pursuant to 37 C.F.R. §1.111 and 1.115  
2/10/97
11. Statement Claiming Small Entity Status  
3/27/97
12. Information Disclosure Statement  
4/10/97
13. Final Office Action  
4/11/97

# PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1994

Application or Docket Number

510133

## CLAIMS AS FILED - PART I

(Column 1)

(Column 2)

FOR	NUMBER FILED	NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	2 minus 20 = *	
INDEPENDENT CLAIMS	3 minus 3 = *	
MULTIPLE DEPENDENT CLAIM PRESENT		

\* If the difference in column 1 is less than zero, enter "0" in column 2.

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	FEE	RATE	FEE
	365.00		730.00
x\$11=		x\$22=	
x38=		x76=	
+120=		+240=	
TOTAL		TOTAL	730

## CLAIMS AS AMENDED - PART II

(Column 1)

(Column 2)

(Column 3)

AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	19 Minus ** 20 =		
Independent	3 Minus *** 3 =		
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

SMALL ENTITY

OR

OTHER THAN SMALL ENTITY

RATE	ADDITIONAL FEE	RATE	ADDITIONAL FEE
x\$11=		x\$22=	
x38=		x76=	
+120=		+240=	
TOTAL ADDIT. FEE		TOTAL ADDIT. FEE	

AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	20 Minus ** 20 = 0		
Independent	2 Minus *** 3 = 0		
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

RATE	ADDITIONAL FEE	RATE	ADDITIONAL FEE
x\$11=		x\$22=	
x38=		x76=	
+120=		+240=	
TOTAL ADDIT. FEE		TOTAL ADDIT. FEE	

AMENDMENT C	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
Total	29 Minus ** 28 = 1		
Independent	4 Minus *** 3 = 1		
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM			

RATE	ADDITIONAL FEE	RATE	ADDITIONAL FEE
x\$11=	54	x\$22=	<
x38=	39	x76=	
+120=		+240=	
TOTAL ADDIT. FEE		TOTAL ADDIT. FEE	

\*\* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.  
 \*\*\* If the "Highest Number Previously Paid For" in THIS SPACE is less than 20, enter "20".  
 If the "Highest Number Previously Paid For" in THIS SPACE is less than 3, enter "3".  
 The Highest Number Previously Paid For (Total or Independent) is the highest number found in the appropriate box in column 1.

## PART B—ISSUE FEE TRANSMITTAL

Complete and mail this form, together with applicable fees, to: **Box ISSUE FEE**  
**Assistant Commissioner for Patents**  
**Washington, D.C. 20231**



**MAILING INSTRUCTIONS:** This form should be used for transmitting the ISSUE FEE. Blocks 1 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

**CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark up with any corrections or use Block 1)**

Marshall, O'Toole, Gerstein, Murray & Borun  
 233 South Wacker Drive  
 63rd Floor  
 Sears Tower  
 Chicago, Illinois 60606

Note: The certificate of mailing can only be used for domestic mailings of the Issue Fee Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing.

## Certificate of Mailing

I hereby certify that this Issue Fee Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Box Issue Fee address above on the date indicated below.

David A. Gagg (Depositor's name)  
 [Signature] (Signature)  
 JANUARY 3, 2001 (Date)

APPLICATION NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
08/510.133	08/01/95	029	SAOUD, C	1047 10/03/00
First Named Applicant: ALITALIA, 35 USC 134(b) Term ext. = 0 Days.				

TITLE OF INVENTION: **THE LIGAND AND METHODS OF USE**  
**FI+4**

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
3 28419/32863 28967 [Signature]	S14-012.000		T33 UTILITY	NO	\$1240.00	01/03/01

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363). Use of PTO form(s) and Customer Number are recommended, but not required.

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.  
☐ "Fee Address" Indication (or "Fee Address" Indication form PTO/SB/47) attached.

2. For printing on the patent front page, list:  
 (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. Marshall, O'Toole,  
 2. Gerstein, Murray  
 3. Borun.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)  
 PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE: **Helsinki University Licensing Ltd. OY; Ludwig Institute for Cancer Research**  
 (B) RESIDENCE (CITY & STATE OR COUNTRY): **Helsinki, Finland; New York, USA**

Please check the appropriate assignee category indicated below (will not be printed on the patent):  
☐ Individual ☒ corporation or other private group entity ☐ government

4a. The following fees are enclosed (make check payable to Commissioner of Patents and Trademarks):

☒ Issue Fee  
☒ Advance Order - # of Copies **4**

4b. The following fees or deficiency in these fees should be charged to:  
 DEPOSIT ACCOUNT NUMBER **13-2855**  
 (ENCLOSE AN EXTRA COPY OF THIS FORM)

☒ Issue Fee  
☐ Advance Order - # of Copies

The COMMISSIONER OF PATENTS AND TRADEMARKS IS requested to apply the Issue Fee to the application identified above.

(Authorized Signature) **[Signature]** (Date) **JAN 03, 2001**

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

**Burden Hour Statement:** This form is estimated to take 0.2 hours to complete. Time will vary depending on the needs of the individual case. Any comments on the amount of time required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND FEES AND THIS FORM TO: Box Issue Fee, Assistant Commissioner for Patents, Washington D.C. 20231

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMIT THIS FORM WITH FEE

PTOL-65B (REV. 10-96) Approved for use through 06/30/99. OMB 0651-0033

Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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 12.00

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[illegible]

APPLICANT/INVENTOR DATA	
UTHORITY CODE	
FAMILY NAME	
GIVEN NAME	
ITY	

IVEN NAME

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AUTHORITY CODE

**AMILY NAME**

**GIVEN NAME**

ITY

NAME SUFFIX

STATE/COUNTRY CODE

NAME SUFFIX

STATE/CTRY CODE

**MORE**

DATE	
DATE	

○

08/510133

FILING DATE: MONTH DAY YEAR: 08 01 95



SPECIAL  
HANDLING

GROUP	ART UNIT	1814
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CLASS

SHEETS OF  
DRAWING

TOTAL CLAIMS	116
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INDEPENDENT  
CLAIMS

**SMALL  
ENTITY?**

**FILING FEE**

FOREIGN  
LICENSE

ATTORNEY DOCKET NUMBER  
681127328103

## CONTINUITY DATA

CONT	STATUS
CODE	CODE
1	1
2	2
3	3
4	4
5	5
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PARENT APPLICATION  
SERIAL NUMBER

PCT APPLICATION SERIAL NUMBER

[illegible]

PARENT FILING  
DATE  
MONTH DAY YEAR

[illegible]

P	C	T	/
P	C	T	/
P	C	T	/
P	C	T	/
P	C	T	/

[illegible]


## PC-T/F-FOREIGN APPLICATION DATA

**FOREIGN  
PRIORITY  
CLAIMED**

COUNTRY  
CODE

PCT/FOREIGN APPLICATION SERIAL NUMBER

FOREIGN  
FILING DATE  
MONTH DAY YEAR

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[illegible]
